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Histopathological and Physiological Responses of *Fundulus heteroclitus* to Naphthalene Exposure[1]

LEONARD DIMICHELE[2]
College of Marine Studies, University of Delaware, Newark, Del. 19711, USA

AND MALCOLM H. TAYLOR
School of Life and Health Sciences, University of Delaware, Newark, Del. 19711, USA

DIMICHELE, L., AND M. H. TAYLOR. 1978. Histopathological and physiological responses of *Fundulus heteroclitus* to naphthalene exposure. J. Fish. Res. Board Can. 35: 1060–1066.

Mummichogs, *Fundulus heteroclitus*, were exposed to naphthalene concentrations ranging from 30 to 0.002 mg/L over time periods ranging from 1 to 15 d. Histopathology of exposed animals was evaluated. Major effects were found in the brain, liver, and pancreas down to 0.2 mg/L for a 15-d exposure. The results of these experiments indicate that the toxicity of naphthalene in this fish is due primarily to its effect on blood components. There was also evidence of neurosensory damage at exposures as low as 0.02 mg/L for 15 d. In addition, serum glucose, protein, and cortisol were measured in animals exposed to low levels of naphthalene for 15 d. All were significantly elevated, indicating metabolic stress at exposures as low as 0.02 mg/L. ^{14}C-naphthalene uptake was measured at two concentrations of naphthalene. Naphthalene was found to accumulate in significant amounts in organs most susceptible to pathology. The distribution of naphthalene was found to be independent of its water concentration in all organs tested except the spleen.

Key words: toxicity, naphthalene, teleosts, histopathology, uptake, *Fundulus*, mummichog

DIMICHELE, L., AND M. H. TAYLOR. 1978. Histopathological and physiological responses of *Fundulus heteroclitus* to naphthalene exposure. J. Fish. Res. Board Can. 35: 1060–1066.

Des choquemorts (*Fundulus heteroclitus*) ont été exposés pendant 1 à 15 jours à des concentrations de naphtalène variant de 30 à 0,002 mg/L en vue d'études histopathologiques. Nous avons pu déceler des effets importants dans le cerveau, le foie et le pancréas, à partir de 0,2 mg/L après 15 jours d'exposition. Nos résultats indiquent que la toxicité du naphtalène découle principalement de ses effets sur les constituants du sang. On a de plus décelé des signes de dommages neurosensoriels à partir d'une exposition à 0,02 mg/L pendant 15 jours. Nous avons aussi mesuré la teneur du sérum en glucose, en protéines et en hydrocortisone, chez les spécimens exposés à de faibles doses de naphtalène pendant 15 jours. Les résultats obtenus étaient tous significativement élevés; des doses de 0,02 mg/L peuvent donc engendrer un stress métabolique. Des mesures de l'absorption du naphtalène marqué au C^{14} à deux concentrations différentes ont démontré une accumulation notable dans les organes les plus sensibles aux effets pathologiques. La répartition du naphtalène dans l'organisme s'est avérée indépendante de la teneur de ce composé en eau pour tous les organes étudiés, sauf la rate.

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POLYCYCLIC aromatic hydrocarbons (PAH) have been cited by several authors as toxic components of crude and refined oil–water mixtures (Anderson et al. 1974a, b; Boylan and Tripp 1971; Neff et al. 1976; Moore and Dwyer 1974). These authors attribute the relatively high toxicity of PAH to their high solubility in water relative to alkanes of similar molecular weight and their rapid uptake and accumulation by marine organisms. PAH are a complex mixture of compounds, and naphthalene and naphthalene derivatives are major compounds taken up by marine organisms. It is thought that naphthalene is among the most toxic of these compounds (Anderson et al. 1974b; Neff et al. 1976).

The toxicity of naphthalene to fish has been demonstrated by Anderson et al. (1974a). They found that the sheepshead minnow (*Cyprinodon variegatus*) concentrates naphthalene 60 times above a water concentration of 1 mg/L in 4 h. Lee et al. (1972) determined

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[2]Present address: School of Life and Health Sciences, University of Delaware, Newark, Del. 19711, USA.

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that naphthalene is selectively accumulated by gills, liver, gut, and gall bladder of the longjaw mudsucker (*Gillichthys mirabilis*) and the tidepool sculpin (*Oligocottus maculosus*). Neff et al. (1976) have obtained similar results for the longnose killifish (*Fundulus similis*) and have shown accumulation of naphthalenes in the brain.

The principal effect of naphthalene poisoning in man has been shown to be hemolytic anemia (Mackell et al. 1951). Gidron and Leurer (1956) have described the major systemic effects of naphthalene poisoning in man to be hemolytic anemia, jaundice, hemoglobinuria, lower nephron nephrosis, and cerebral anoxia caused by blockage of the capillaries. All of these symptoms are presumably secondary effects of localized, chemically induced hemolysis.

The purpose of these experiments was to document specific histological and physiological effects of continuous low-level exposures to naphthalene in *F. heteroclitus*. Histological effects were documented to assess significant pathologies caused by naphthalene exposure. Hematocrits, and serum cortisol, glucose, and protein, were measured as evidence of metabolic stress. ^{14}C-naphthalene uptake was determined in order to relate observed pathologies with naphthalene distribution patterns among body organs.

Materials and Methods

Fish used in this study (*Fundulus heteroclitus*) were collected from the Canary Creek salt marsh, Lewes, Delaware, during September and October 1975. Upon return to the laboratory, the fish were acclimated for 1 mo in saltwater aquaria (Instant Ocean) at 15‰ salinity and a temperature of 20 ± 1.0°C (SE). During this time, the fish were maintained on a diet of Purina Trout Chow (Ralston-Purina Corp.), ad lib., and frozen fish twice a week. The light cycle was maintained at 14:10 (light:dark) throughout the acclimation period and during the experiments.

The toxicity experiments were carried out in 76-L aquaria equipped with undergravel recirculating filters and maintained at the above salinity and temperature. The acclimation period was continued for one additional week after transfer to the experimental aquaria. Temperature and salinity were maintained during the experiment at the levels established for the acclimation period. The mean dissolved oxygen was 8.3 ± 0.2 mg/L, measured by an oxygen meter (Yellow Springs Inst. Co.). Mean pH was 7.6 ± 0.2, and ammonia was 0.01 ± 0.02 mg/L as determined by the method of Strickland and Parsons (1968).

Naphthalene was added to the water via 1 mL of 80% ethanol stock solutions of varying concentration. Naphthalene concentrations were determined by the method of Neff and Anderson (1975) modified to use a 5-mL sample and omitting the florasil adsorbant. Measurements were made daily in short-term exposures, and biweekly in long-term exposures. The rate of naphthalene loss from the water was determined for each tank during the first several hours of exposure and was used to calculate additions for maintenance of exposure level. The exposure concentration was allowed to vary 5% between additions. While our method of addition proved to be highly reproducible, several aquaria developed bacterial populations (pseudomonads) which removed naphthalene and dissolved oxygen from the water. This problem occurred more frequently in aquaria containing naphthalene concentrations above 2 mg/L.

For each experiment, 10 fish were chosen from stock aquaria for exposure to each test concentration. An additional 20 fish were selected to serve as controls; 10 of these received 1 mL of 80% ethanol per addition of naphthalene to the experimental aquaria and 10 received no treatment. The mean length of the fish used was 8.2 ± 0.8 cm. The sex ratio of experimental animals was 2.1:1 (female:male). Analysis of variance of the sex ratio showed no significant differences among the tanks ($P < 0.05$). At the time of the experiments, fall 1975, all of the fish were sexually regressed.

The first experiment was a 24-h exposure with test concentrations of 30, 20, and 10 mg naphthalene/L; the second, a 96-h study at 8, 4, and 2 mg naphthalene/L; and the third, a 15-d exposure at 2.0, 0.2, and 0.002 mg naphthalene/L. In all experiments feeding was discontinued 24 h before the fish were killed. Moribund fish were removed immediately when they floated "belly up" and opercular movements ceased. Blood samples from moribund fish and all others at the termination of the experiment were collected according to the method of Pickford et al. 1969. Sampling was completed within 3 min after removal of fish from the aquarium. The fish were then fixed for an initial period of 15 min in Dietrich's solution, cut into tissue blocks, and fixed overnight in the same solution. Subsequent paraffin embedding and staining procedures were as described by Thompson (1966). Several sections of each block were cut at 6 μm and stained with hematoxylin and eosin. Tissue sections were examined under a bright-field microscope. Observed pathologies were compared to control fish and fish collected from the field.

Blood samples were centrifuged at 2000 rpm for 15 min in capillary tubes. Hematocrit values were obtained by measuring the ratio of cellular length to total length of the sample in the tube. The serum fractions were stored frozen in polyethylene vials. Serum cortisol was measured by a radioimmunoassay procedure. Reagents were prepared as described by the enclosures provided with the antibody (F-21-53, Endocrine Sciences, Tarzana, California). The procedure was modified slightly to permit the use of a 5-μL serum sample. Cross-reactivity of corticosterone relative to cortisol was 35%; of cortisone, 10%; testosterone, 4%; deoxycorticosterone, 3%; and progesterone, 3%. Similar results were obtained by Krey et al. (1975) using antibody from the same supplier.

An estimate of the recovery of cortisol by the assay was determined using a 5-μL control serum sample containing 0.5 ng of known cortisol standard. The mean recovery of 12 samples was 0.48 ± 0.08 ng, an overall recovery of 96%. Precision of the assay procedure was calculated to be 5.4% (coefficient of variation) in five separate triplicate determinations of a pooled serum sample of mean cortisol concentration 8.6 μg% (Leach and Taylor 1977).

A glucose oxidase procedure was used for serum glucose determinations (Worthington Biochemical Corporation, Freehold, New Jersey). Total serum protein determinations were made by the method of Lowry et al. (1951). The procedure was modified to use 1 μL of sample. Reported protein concentrations are measured as protein equivalents of bovine serum albumin.

In the uptake experiment six fish were tested at each of two concentrations of naphthalene (2 and 0.02 mg/L) and

TABLE 1. Pathologies of fish exposed to naphthalene. Subscript E: extensive pathology, usually involves large areas; subscript F: focal pathology, usually involves small areas; no subscript: pathologies focal but infrequent.

	Naphthalene in test water (mg/L)										
	30	20	10	8	4	2	2	.2	.02	.002	controls
Exposure time (d)	1	1	1	4	4	4	15	15	15	15	1/4/15
Pathology	% of animals										
Mortality	100	100	0	100	10	0	80	0	0	0	0
Gill hyperplasia	100_E	100_E	100	100	100	80	100_E	100	100	80	30
Gill hemorrhage	100_E	100_E	80	30	0	0	50	0	0	0	0
Tastebud necrosis	100_E	100_E	100_E	100_E	100_E	100	100_E	100	100	0	0
Nasal and lateral line necrosis	100	100	90	30	0	0	100	100	20	0	0
Pancreatic ischemia	100_E	100_E	100_F	100_F	100_F	0	100_E	100_F	0	0	0
Hepatic ischemia	100_F	100_F	100_F	100_E	100_F	0	100_E	100_F	0	0	0
Cerebral ischemia	100_E	100_E	100	100_E	80	0	100_E	100	0	0	0
Renal ischemia	0	0	0	100_E	50	0	100_E	30	0	0	0
Hypertrophy of interrenal cells	0	0	0	100	100	100	100_E	100	0	0	0
Necrosis of interrenal cells	0	0	0	0	0	0	100_E	0	0	0	0
Necrosis and sloughing of the intestinal mucosa	100_E	100_E	0	0	0	0	30_F	0	0	0	0
Muscle necrosis	0	0	0	0	0	0	100	100	0	0	0

six fish served as controls. Pairs of fish were placed in 3.8-L jars containing 2 L of water (15‰ salinity) of the appropriate naphthalene concentration with 10 μCi of ^{14}C-naphthalene added as a tracer. The fish were exposed to naphthalene for 4 h, then rinsed in methanol to remove any naphthalene on the body surface, and killed as described below.

Three fish from each group were dissected and the organs to be assayed for naphthalene (liver, brain, head kidney, spleen, gall bladder, gut, gill, and heart) weighed and homogenized in 2 mL of toluene. The remaining three fish in each group were homogenized in a Waring blender with 50 mL of toluene. The extraction medium was then centrifuged at 15 000 rpm for 5 min to remove particulate matter. Aliquots of the supernatant of those fish exposed to naphthalene were then placed in 5 mL of scintillation fluid (POPOP). Known amounts of ^{14}C-naphthalene dissolved in toluene were added to the supernatant of control fish to determine quenching. Blood serum samples from the three dissected fish were added directly to the scintillation fluid. All scintillation vials were then shaken for 5 min and counted in a liquid scintillation counter (Beckman LS100). Second extractions of tissue samples revealed no further extractable radioactivity. Reported values of naphthalene concentrations in tissues were calculated from the specific activity (microcuries per gram naphthalene) of the exposure medium.

Data from serum samples and the uptake experiment were analyzed by Duncan's multiple range test. All data were tested for significance at $P < 0.05$.

Results

Histological examination of exposed animals revealed some pathology at all naphthalene concentrations tested. The experimental animals were divided into 10 groups based on exposure concentration and time. There were no differences between the control groups that received ethanol and those that received no treatment; therefore, all controls were combined in an 11th group for analysis. A summary of results is given in Table 1.

Gill hyperplasia occurred in 30% of the controls and at all naphthalene concentrations tested. Hemorrhages of the gill filaments were also prevalent. Animals exposed to lethal acute concentrations of naphthalene showed extensive hemorrhagic areas. In general, hemorrhages were observed most frequently near the base of the gill filaments.

Neurosensory cells were affected at all exposures except 0.002 mg/L for 15 d. Neurosensory cells showing early signs of necrosis had long spindle-shaped nuclei. Completely necrotic taste buds were often collapsed empty spots in the epithelium of the lips, mouth, and pharynx. Figures 1 and 2 illustrate pathologies typical for the olfactory organ and lateral line system.

Evidence of blood stasis was present in all pathologic tissues noted in these experiments except in sensory organs. In extensively damaged areas of the pancreas, liver, and brain, small blood vessels were engorged with cells, while similar vessels in control fish contained few to no cells. Where necrosis was not severe, lesions were small spherical areas scattered throughout the organ, suggesting a reduced oxygen supply due to a blocked vessel. Extensive pancreatic necrosis was evident in animals exposed to 30 and 20 mg/L for 1 d and 2 mg/L for 15 d, while animals exposed to 10 mg/L for 1 d, 8, 4, and 2 mg/L for 4 d, and 0.2 mg/L for 15 d showed only focal necrosis. Pancreatic necrosis often included the islet tissue in the mesenteric pancreas. Lesions found in the liver followed much the same pattern as in the pancreas except fish exposed to naphthalene for 1 d had only focal liver lesions.

Fish exposed to 30 and 20 mg/L for 1 d, 8 mg/L for 4 d, and 2 mg/L for 15 d exhibited extensive cerebral

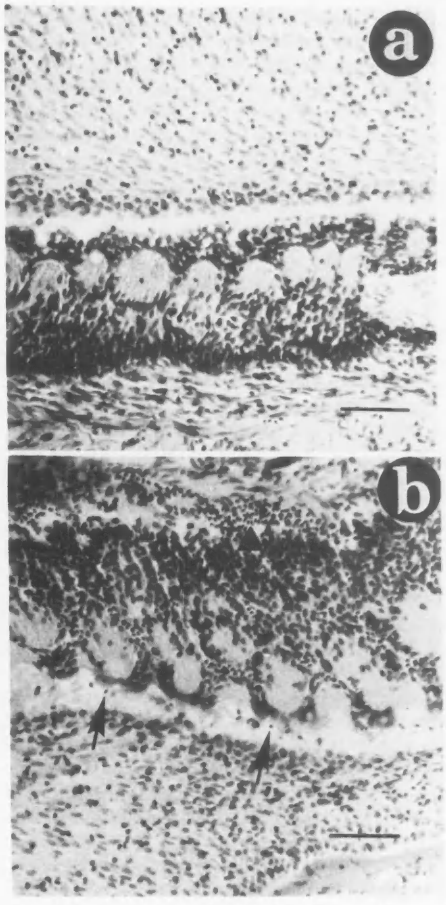

FIG. 1. Olfactory organ of *Fundulus heteroclitus*. a, Control; b, after exposure to 0.2 mg/L naphthalene for 15 d. Note the necrosis and sloughing of cells into the lumen, the pyknotic nuclei (arrows), and the lymphocytic infiltration (triangles). Bars. = 40 μm.

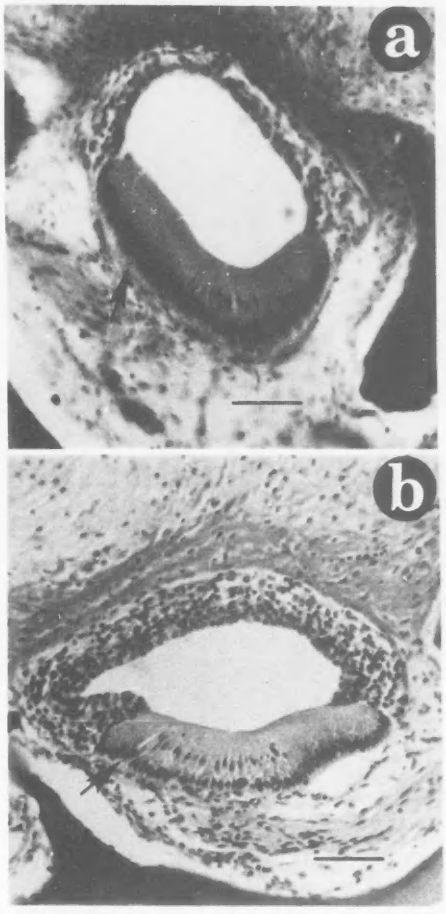

FIG. 2. Mandibular canal and neuromast. a, Control; b, after exposure to 8.0 mg/L naphthalene for 4 d. Note the collapse of sustentacular cells (arrows). Bars = 40 μm.

ischemia; those exposed to 10 mg/L for 1 d, 4 mg/L for 4 d, and 0.2 mg/L for 15 d showed signs of focal congestion in cerebral vessels (Fig. 3).

Renal tissue was unaffected in animals exposed to 30, 20, and 10 mg/L for 1 d. Animals exposed to 8 mg/L for 4 d and 2 mg/L for 15 d exhibited an eosinophilic exudate in the proximal and distal collecting tubules, as well as ischemia and hemostasis. Renal tissue in animals exposed to 0.02 and 0.002 mg/L for 15 d was unaffected. Animals exposed to 8, 4, and 2 mg/L for 1 d and 2 and 12 mg/L for 15 d showed a marked hypertrophy of interrenal cells and in those exposed to 2.0 mg/L for 15 d most of the interrenal cells were necrotic.

Lesions of the gut were confined to the mucosa. Necrosis and sloughing of the mucosa occurred only in those fish severely affected by the toxicant. Animals exposed to 30 and 20 mg/L for 1 d displayed extensive sloughing throughout the gut, while in animals exposed to 2 mg/L for 15 d the condition occurred in only three of the fish. Furthermore, lesions occurred throughout the gut in discrete foci only.

Fish exposed to 2 and 0.2 mg/L for 15 d showed distinct signs of muscle degeneration. The muscle tissue of all other animals was unaffected.

Hematocrit values among the experimental groups showed no significant difference from control values except for those fish exposed to 8 mg/L for 4 d. Hematocrits from this group (28.5 ± 3.0%, SE) were lower than those of controls (35.7 ± 3.0%).

All of the serum variables measured showed a response to naphthalene exposure (Table 2). Serum cortisol concentrations were proportional to the

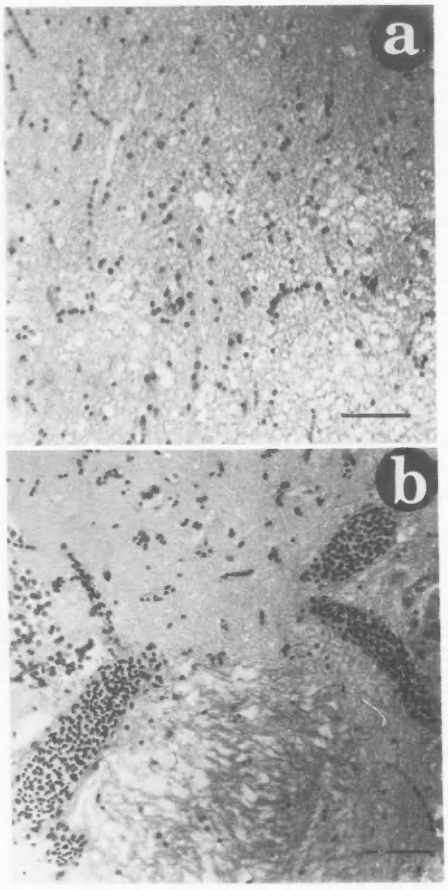

FIG. 3. Brain. a, Control; b, ischemic area of the brain after exposure to 20 mg/L for 6 h. Note the congestion in blood vessels. While control brain tissue stains red with H & E, affected brain tissue stains faint blue. Bars = 40 μm.

naphthalene concentration in the water between 0.2 and 0.002 mg/L. The average control cortisol concentration was high compared to normal unstressed fish. The abnormal values obtained are discussed later in this paper. Glucose and protein showed a similar response. In each case, the fish exposed to the three highest concentrations of naphthalene tested showed a significant elevation as compared to controls.

Table 3 summarizes the results of the ^{14}C-naphthalene uptake experiment. Analyses of whole fish indicated that *F. heteroclitus* accumulated naphthalene to approximately 2 times above environmental levels in 4 h, at water concentrations of 2 and 0.02 mg/L. The toxicant was not uniformly distributed among the organs assayed. Analysis of variance showed that the concentration factors for each organ at the two test concentrations were significantly different ($P < 0.05$) only in the case of the spleen. The radioactivity found in the spleen accounts for $50 \pm 5\%$, SE of the total radioactivity in animals exposed to 2 mg/L, whereas it accounts for only $16 \pm 2\%$ of the total radioactivity found in those animals exposed to 0.02 mg/L. Naphthalene assayed in organs tested accounted for $79.3 \pm 8\%$ of the total naphthalene in each animal, although these organs account for only $13.5 \pm 4\%$ of the animals' weight.

Discussion

Histological evidence from these experiments indicates two major responses of *F. heteroclitus* to naphthalene intoxication. In fish continuously exposed to doses above 2 mg/L for 15 d there appears to be a generalized blood stasis. At doses as low as 0.02 mg/L for 15 d there is a degeneration of neurosensory organs, apparently unrelated to the hemostasis. A dose of 0.002 mg/L naphthalene for 15 d produced no significant pathologies.

Evidence presented here indicates that the blood effects of naphthalene poisoning in *F. heteroclitus* are similar to the mammalian case, a stasis that results in ischemia of several tissues. It is not clear from these experiments whether the stasis is due to a hemolytic crisis as in mammals or to some other process. Only one group of animals, those exposed to 8 mg/L for 4 d, showed a reduced hematocrit. Virtually all forms of hemolytic anemia stem from alterations in the erythrocyte membrane, usually in the form of an increased rigidity of the membrane. In chemically induced hemolytic anemias of mammalian systems, hemolysis occurs as a result of rigid cells lodged in capillaries bursting under the stress exerted by arterial pressures (Jacob 1975). The relatively low blood pressures present in the circulatory system of fish may be insuffi-

TABLE 2. Serum cortisol, glucose, and protein in *F. heteroclitus* after 15-d exposure to naphthalene. Each value is an average of 10 fish ± SE. Values followed by similar superscripts are not significantly different by Duncan's multiple range test ($P < 0.05$).

	Naphthalene in test water (mg/L)				
	2.0	0.2	0.02	0.002	Control
Serum cortisol (μg/100 mL)	8.9 ± 1.0^{A}	22.9 ± 2.3	7.0 ± 1.8^{A}	0.68 ± 0.3	13.8 ± 2.7
Serum glucose (mg/100 mL)	121.0 ± 5.9	79.4 ± 2.0^{A}	75.8 ± 2.0^{A}	64.2 ± 4.4^{B}	63.4 ± 2.4^{B}
Serum protein (g/100 mL)	10.5 ± 0.8	8.0 ± 0.5^{A}	8.4 ± 0.5^{A}	7.2 ± 0.6^{AB}	6.4 ± 0.2^{B}

TABLE 3. Naphthalene uptake by *F. heteroclitus* after 4 h of continuous exposure. Each value is an average of three fish ± SE. Values followed by similar superscripts are not significantly different by Duncan's multiple range test ($P < 0.05$).

Naphthalene in test water (mg/L)	Naphthalene in tissues (μg/g fresh weight)[a]									
	Whole fish	Spleen	Head kidney	Heart	Brain	Liver	Gall bladder	Gut	Gill	Blood
0.02	0.04±0.02^{C}	0.68±0.01	0.35±0.03	0.29±0.01^{A}	0.3±0.01^{A}	0.29±0.01^{A}	0.18±0.01	0.17±0.01	0.050±0.004^{C}	0.020±0.015
(C.F.)[b]	2.0±0.2	34.0±0.3	17.5±0.5	14.5±0.3	15.0±0.3	14.5±0.3	9.0±0.3	8.5±0.3	2.7±0.2	1.0±0.3
2.0	4.4±0.7^{C}	210.0±7.6	44.0±1.0	37.0±0.33^{A}	32.0±1.5^{AB}	27.0±1.0^{B}	13.0±1.5^{D}	14.0±1.5^{D}	5.9±0.2^{C}	2.0±0.1
(C.F)	2.2±0.0	105.0±2.8	22.0±0.5	18.5±0.2	16.0±0.8	13.5±0.5	6.7±0.9	7.0±0.9	3.0±0.0	1.0±0.0

[a]Blood concentration is given in milligrams per litre.
[b]C.F.: concentration factor above naphthalene concentration in test water.

$$\text{C.F.} = \frac{\mu\text{g naphthalene/g tissue}}{\mu\text{g naphthalene/g sea water}}$$

cient to break erythrocytes lodged in the capillaries. The result would be a simple log-jamming effect rather than hemolysis. Sabo and Stegeman (1977) have reported evidence that petroleum hydrocarbons affect membrane structure in *Fundulus*.

Gill hyperplasia was the most consistent lesion that occurred in the study. It was seen in nearly every exposed animal. Hyperplasia of the gill epithelium is commonly seen in fish exposed to almost any irritant (Gardner 1975). Since it commonly occurs as a result of parasitic infection, it is unlikely to be a reliable indicator of chemically induced stress.

Lesions of the sensory systems, however, may prove to be a valuable diagnostic tool. Taste buds, lateral line sensory cells, and the olfactory epithelium appeared to be sensitive indicators of chronic exposure in this study. Neurosensory cells also have been shown to react similarly in other toxicant systems. Gardner (1975) has reported neurosensory disorders in fish exposed to copper, mercury, silver, methoxychlor, and crude oil. Full assessment of the impact of a pollutant requires a sensitive and reliable indicator relevant to the continued survival of aquatic animals (Gardner 1975). Histology of sensitive tissues, such as the olfactory and lateral line organs of fish, can provide a simple, fast, and semiquantitative bioassay procedure in laboratory experiments and receiving waters (Marine Bioassays Workshop 1974).

Many teleosts respond to stress with the secretion of glucocorticoids (Jurani et al. 1972; Redgate 1974; Singley and Chavin 1975). Serum cortisol levels in the 15-d experiment exhibited a dose–response relationship with the exception of the 2 mg/L group and controls. In our laboratory, basal cortisol levels range from 0 to 5 μg% (Leach and Taylor 1977). Histological examination of the interrenal cells of controls indicated no hypertrophy of these cells and the serum levels of glucose and protein were normal. For these reasons, we believe the abnormally high control levels were due to a transient activation of interrenal secretion during the final sampling. The controls were killed after the experimental groups, and the physical activity around the tanks may have been sufficient to cause a rise in cortisol levels.

Cortisol levels were markedly lower for the 2 mg/L group than for the next highest exposure group (0.2 mg/L). This may have been due to the severe necrosis of interrenal tissue found in the 2 mg/L group. Serum cortisol, glucose, and protein levels in these experiments indicate an activation of metabolic systems in response to naphthalene concentrations in the water as low as 0.02 mg/L.

In general, those organs most sensitive to pathology accumulated high concentrations of naphthalene. Furthermore, comparisons of the concentration factors indicated that the pattern of naphthalene distribution among the organs is independent of the water concentration, except for the spleen. The proportionally larger accumulation in the spleens of fish exposed to 2.0 mg/L may have been due to accumulations of dead erythrocytes containing naphthalene.

In summary, our results indicated that the toxicity of naphthalene in *F. heteroclitus* is due primarily to its effects on blood components in lethal acute situations. In sublethal situations there is evidence of neurosensory damage and metabolic stress which may affect the animal's overall ability to survive in the natural environment.

Acknowledgments

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Circulation, Variability, and Dynamics of the Scotian Shelf and Slope

PETER C. SMITH, BRIAN PETRIE, AND C. R. MANN

Department of Fisheries and the Environment, Fisheries and Marine Service, Atlantic Oceanographic Laboratory, Bedford Institute of Oceanography, Dartmouth, N.S. B2Y 4A2

SMITH, P. C., B. PETRIE, AND C. R. MANN. 1978. Circulation, variability, and dynamics of the Scotian Shelf and Slope. J. Fish. Res. Board Can. 35: 1067–1083.

A brief summary of historical data relating to the mean circulation on the Scotian Shelf and Slope reveals the distribution of various water masses and certain general characteristics of the current field, supported by geostrophic calculations and observations of surface drift. However, extreme variability resulting from wind and tidal forces is evident in all measurements made on the shelf and slope. Various measurements and models of the first-order variable flows, including wind-driven circulation, diurnal and semidiurnal tides, and inertial waves, are also reviewed. Results from a new model for inertial motions in a variable-depth mixed layer are found to closely reproduce the transient behavior of inertial energy at the 50-m depth on the shelf, but certain aspects of the model are inconsistent with the observations. With results from a mooring program on the shelf in September–December 1968, the dynamics of the mean circulation are examined in the context of a vertically integrated, two-dimensional model. Reynolds stresses associated with the first-order flows are found to be insignificant in the inshore zone (within 90 km of the coast) where the balances are between mean wind and bottom stresses, surface pressure gradients, and Coriolis forces. Over the outer shelf, however, the geostrophic onshore balance breaks down and Reynolds stress divergences in the inertial and tidal bands become important. At the shelf break, generation of the baroclinic semidiurnal tide produces internal Reynolds stress forces comparable to the barotropic terms on the shelf.

Key words: mean circulation, semidiurnal barotropic and internal tides, inertial waves, mixed layer, wind-driven circulation, Reynolds stress, geostrophic, Scotian Shelf

SMITH, P. C., B. PETRIE, AND C. R. MANN. 1978. Circulation, variability, and dynamics of the Scotian Shelf and Slope. J. Fish. Res. Board Can. 35: 1067–1083.

A l'aide d'un bref résumé des données existantes concernant la circulation moyenne de l'eau sur le plateau et le talus continentaux Scotian, les auteurs donnent la répartition des diverses masses d'eau et certaines caractéristiques générales du champ du courant, appuyées par des approximations géostrophiques et des observations de la dérive à la surface. Les mesures prises sur le plateau et le talus sont cependant toutes extrêmement variables à cause des forces du vent et des marées. Les auteurs passent aussi en revue des mesures et des modèles des mouvements variables d'ordre un, tenant compte de la force d'entraînement du vent, des marées diurnes et semi-diurnes, ainsi que des ondes d'inertie. L'application d'un nouveau modèle du mouvement d'inertie d'une couche mixte à profondeur variable semble reproduire fidèlement le comportement transitoire de la force d'inertie sur le plateau, à 50 m de profondeur, bien que quelques aspects du modèle ne soient pas conformes aux observations. Grâce aux résultats d'ammarages effectués sur le plateau entre septembre et décembre 1968, il leur a été possible d'étudier la dynamique de la circulation moyenne à l'aide d'un modèle à deux dimensions, intégré dans le sens vertical. Les tensions de Reynolds liées au mouvement d'ordre un sont presque inexistantes dans la zone littorale (jusqu'à 90 km de la côte), où l'équilibre s'établit entre les vents moyens et les tensions sur le fond, entre les gradients de la tension superficielle et les forces de Coriolis. Plus loin sur le plateau, cependant, l'équilibre géostrophique du littoral se rompt et les divergences engendrées par les tensions de Reynolds dans les ondes d'inertie et des marées deviennent importantes. Au pied du plateau, les marées baroclines et semi-diurnes produisent des forces internes, dues aux tensions de Reynolds, analogues à ce que créent les composantes barotropes du talus continental.

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THE continental margin south of Nova Scotia comprises the Scotian Shelf and Slope. The Scotian Shelf, bounded by the Laurentian Channel to the east and the Northeast Channel to the west, is ~700 km long and 160–240 km wide with an average depth of 90 m. The highly irregular bottom relief on the shelf is characterized by deep basins at midshelf and shallow banks in the outer portions.

Early oceanographic observations on the Scotian Shelf and Slope were commissioned by the Fisheries

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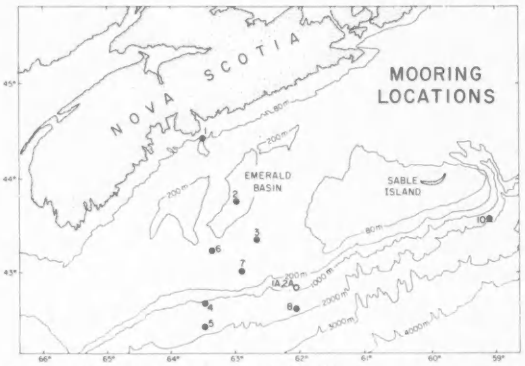

FIG. 1. Mooring sites for the 1967–68 current measurement program.

Research Board of Canada (and its forerunners) and consisted mainly of standard oceanographic sections and drift bottle surveys (e.g. Bjerkan 1919; Hachey 1942; McLellan 1954, 1957; Trites and Banks 1958). In 1950 a series of standard hydrographic sections along the coast of Nova Scotia was initiated and an attempt made to occupy them seasonally for about a decade. Subsequently, only one of the sections continued to be occupied and became known as the "Halifax Section." It consists of at least eight stations stretching from Halifax to the continental slope. Hydrographic section data for the entire region since 1950 are contained in reports by Taylor (1961, 1966) and de la Ronde (1972).

Data from these surveys provide a reasonable description of gross seasonal changes in the water mass distribution and surface drift on the Scotian Shelf and Slope. Furthermore, the continuity of these early results gives the impression of a coherent mean circulation over the region. However, as the spatial and temporal resolution of hydrographic measurements continued to improve, the extreme variability of the shelf and slope waters has become increasingly evident. In summarizing Halifax Section variability, Mann (1969) pointed out that the aliasing of such data by fluctuations at periods of a week or less will severely degrade measurements of mean conditions and suggested that moored, rapid-sampling sensors be used to assess the importance of the higher frequencies. In 1967–68, a 2-yr program of current meter and thermograph measurements over the Scotian Shelf and Slope was conducted (see Fig. 1 for the primary mooring sites, Stations 1–8, 10). The data found in reports by Grant and Reiniger (1970, 1971), form the basis for a series of comprehensive investigations by students at Dalhousie University. Warner (1970) made a general study of water movement on the Scotian Shelf, while Lee (1970) and Gatien (1976) concentrated on circulation and mixing in the slope water. Petrie (1974, 1975) analyzed tidal motion on the shelf and slope; his investigation included a small-scale mooring experiment on the continental slope (Fig. 1, Stations 1A, 2A).

Recently, Petrie and Smith (1977) have examined low-frequency content of the 1967–68 data. They find high current variance in response to wind at periods from 2.5 to 7.0 d and also some lower frequency motion, particularly over the slope, which may be related to deep ocean forcing. From these studies, it is clear that the main driving forces on the Scotian Shelf and Slope are tides, wind, and possibly momentum exchange with the deep ocean. Hence the problem of determining the mean circulation (observationally or theoretically) becomes one of extracting a weak residual flow from a strongly varying background.

From a modeling viewpoint, the dominance of the variable first-order flow provides certain simplifications to the mean flow momentum balances. Csanady (1976) has proposed a model for the mean circulation on the continental shelf in which the flow is governed by the hydrostatic and Boussinesq approximations to the Navier–Stokes equations and which includes some Reynolds stress terms representing the nonlinear advection of momentum by the first-order flow, i.e. the response to atmospheric and tidal forces. The theoretical basis for the present study is a similar set of equations:

$$U_x + V_y + W_z = 0, \tag{1}$$

$$-fV = -g(\zeta + D)_x - (\overline{u'^2})_x - (\overline{u'v'})_y - (\overline{u'w'})_z, \tag{2}$$

$$fU = -g(\zeta + D)_y - (\overline{u'v'})_x - (\overline{v'^2})_z - (\overline{v'w'})_z, \tag{3}$$

where the subscript implies differentiation; the overbar represents the average over a time, T, much greater than atmosphere or tidal periods; (U, V, W) and (u', $'v'$, $'w'$) are the mean and first-order flow components; ζ is the deviation of the sea surface about the mean level ($z = 0$); $D = \int_z^0 (\Delta\rho/\rho_0)\, dz$ is the dynamic height based on the density perturbation, $\Delta\rho = \rho - \rho_0$ (ρ_0 = surface density) and f and g are the Coriolis parameter and gravity. In the following analyses, the origin of coordinates is taken at the edge of the shelf with y onshore and x alongshore to the east. These equations differ from Csanady's by the inclusion of Reynolds stresses acting on vertical [e.g. $(\overline{u'v'})$] as well as horizontal [$(\overline{u'w'})$] planes. Csanady assumes that the former are unimportant outside of the coastal boundary layer and neglects them. One of the purposes of this work is to test that hypothesis with empirical evidence from the Scotian Shelf.

The primary objectives of this paper are twofold: (1) to review present understanding of the circulation and variability on the Scotian Shelf and Slope, and (2) to examine the momentum balance for the mean circulation on the Shelf. In the next section, the important mean quantities in the momentum equations are brought out in a review of historical data relating to the general circulation. Using simple dynamical models where possible, the third section examines the major sources of variability and evaluates some of the first-order momentum fluxes on the shelf. Most of this section constitutes a review of published work except for the modeling of inertial motions. In the fourth and fifth sections, the various contributions to the mean momentum balance are compared and discussed.

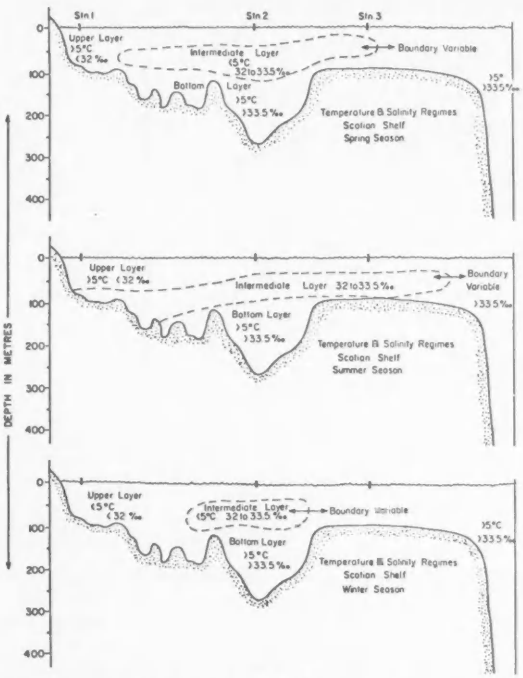

FIG. 2. Approximate boundaries for three-layer system on the Scotian Shelf at Halifax in three seasons (from Warner 1970).

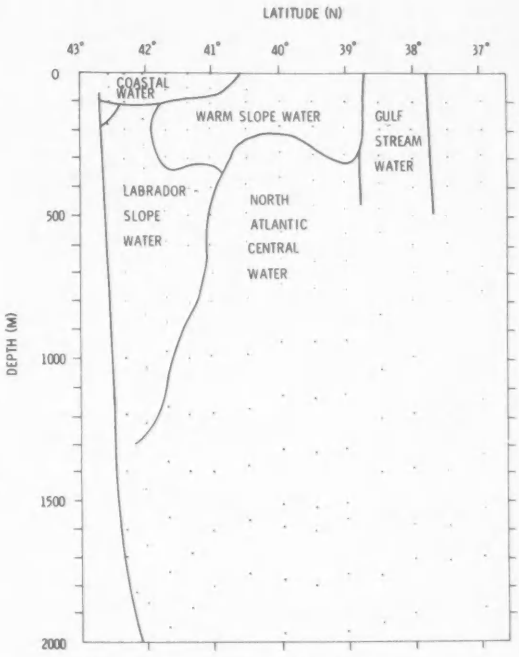

FIG. 3. Approximate boundaries in the slope water regime along 64°30′W during the Gulf Stream '60 survey (from Gatien 1976).

Mean Conditions

Waters of the Scotian Shelf and Slope are derived from three main sources: runoff from the Gulf of St. Lawrence, the Labrador Current, and North Atlantic water. Hachey (1942) viewed the water masses on the Scotian Shelf as forming a three-layer system: (a) a surface layer with salinity <32‰ and seasonal temperatures (>5°C in summer, <5°C in winter); (b) a cold intermediate layer with salinities of 32–33.5‰ and temperatures <5°C; and (c) a warm saline bottom layer with salinities >33.5‰ and temperatures >5°C. The extent of these layers over the shelf at Halifax is sketched in Fig. 2 for three seasons (Warner 1970). The three-layer system is usually well established from late spring to early autumn when a strong pycnocline develops between the warm surface and cold intermediate layers. However, in winter the water in inshore areas and over the shallow banks is often well-mixed to the bottom or consists of only two layers.

Analysis of T–S (temperature–salinity) characteristics of the shelf waters (McLellan 1954) indicates that the upper layer consists primarily of surface water from the Gulf of St. Lawrence which is mixed with an increasing proportion of surface slope water as it flows westward. Similarly, Labrador Current water, which appears as a thick layer in Cabot Strait, is considerably modified by slope water as it floods the shelf to the west. Thus advection, rather than in situ winter cooling, is responsible for the presence of the cold intermediate layer on the shelf (Hachey 1938). The T–S properties of water in the bottom layer are similar to those of the slope water impinging on the outer banks which implies direct communication between the two. Quantitatively, a recent investigation by Houghton et al. (1978) has shown that low-frequency shoreward diffusion of heat and salt in this layer are consistent with measured longshore gradients of temperature and salinity on the shelf in the context of a simple box model. However, in certain areas submarine channels and gulfs through the outer banks enhance this exchange of deep shelf and slope waters, while in others, shallow banks limit the penetration of offshore waters and prevent the exchange of deep waters of the northeast and southwest portions of the shelf (Hachey 1942).

Controversies concerning the origin and extent of slope water masses have only recently been resolved by Gatien (1976), who demonstrated the existence of two distinct water types within the slope water regime. A diagram of their general distribution in a section south of Halifax is presented in Fig. 3. At the surface and between the fresh coastal water and the Gulf Stream lies a body of warm, saline slope water in which temperature and salinity increase to the east. Below 200 m an incomplete mixture of Labrador and North Atlantic Central Water is pressed against the continental slope. In this regime, temperature and salinity decrease to the east. Gatien referred to these

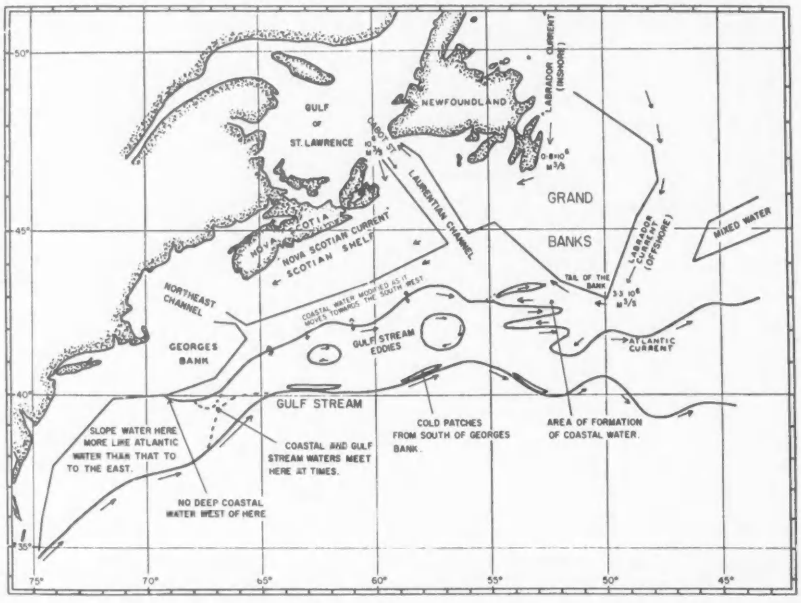
GULF OF ST. LAWRENCE
NEWFOUNDLAND
LABRADOR CURRENT (INSHORE)
GRAND BANKS
LABRADOR CURRENT (OFFSHORE)
MIXED WATER
LAURENTIAN CHANNEL
NOVA SCOTIAN CURRENT
NOVA SCOTIAN SHELF
NORTHEAST CHANNEL
GEORGES BANK
GULF STREAM EDDIES
GULF STREAM
ATLANTIC CURRENT
TAIL OF THE BANK
SLOPE WATER HERE MORE LIKE ATLANTIC WATER THAN THAT TO TO THE EAST.
COASTAL AND GULF STREAM WATERS MEET HERE AT TIMES.
NO DEEP COASTAL WATER WEST OF HERE
COLD PATCHES FROM SOUTH OF GEORGES BANK.
AREA OF FORMATION OF COASTAL WATER.

FIG. 4. A model for the general circulation in the shelf and slope waters (from McLellan 1957).

water masses as Warm Slope Water and Labrador Slope Water and distinguished them from North Atlantic water using sections of dissolved oxygen and salinity anomalies.

Recent hydrographic evidence suggests that the sharp northern boundaries (often <1 km across) of the slope water regime are regions of intense interleaving between shelf and slope water masses. Double diffusion has been suggested as a mechanism for the strong mixing believed to occur in these zones (Horne 1978). On a larger scale, these boundaries are highly convoluted and nonstationary. McLellan et al. (1953) pointed out that the well-defined surface slope water boundary varies "unsystematically" by as much as 250 km south of Halifax, while the width of the slope water region fluctuates from 30 to 220 km. In addition, Gulf Stream eddies and detached parcels of shelf water are regularly found within the slope water.

Using conservation principles, McLellan (1957) was able to construct a crude model for the general circulation of the entire region from hydrographic data (Fig. 4). Assuming that mixing occurs on constant potential density surfaces, he concluded that columns of North Atlantic water were mixing with coastal and Labrador water in ratios of 4:1. Inshore and offshore branches of the Labrador Current enter the region from the east with transports of 0.8×10^6 and 3.3×10^6 m^3·s^{-1}, respectively. The Nova Scotian Current, carrying 1.0×10^6 m^3·s^{-1} out of Cabot Strait, is nearly compensated by influx into the Gulf of St. Lawrence on the eastern side of the Strait, but augmented slightly by contributions from the St. Lawrence River and possibly flow through the Straight of Belle Isle. In addition, 16.0×10^6 m^3·s^{-1} of Atlantic water must be entrained to give the proper constituent ratios in the slope water. To balance these inputs, an eastward transport of 20.0×10^6 m^3·s^{-1} is required at the Tail of the Bank. Gatien's (1976) analysis produced substantially the same result, except that she designated deep westward flow just off the shelf as "Labrador Slope Water" rather than "Coastal Water."

McLellan's estimate of the eastward flow in the slope water has been confirmed in geostrophic calculations at the Tail of the Bank by Foote et al. (1965), who measured a transport of 15×10^6 m^3·s^{-1} between the surface and 2000 m. The surface velocity in the slope water, as determined by GEK (geomagnetic electrokinetograph) measurements (McLellan et al. 1953; Foote et al. 1965), has distinct maxima at the northern and southern boundaries of the region. The northern branch, which coincides with the slope water boundary, is somewhat diffuse but unmistakable south of Halifax and intensifies to 20–30 cm·s^{-1} at the Tail of the Bank. Despite strong contrasts of T and S, there was no detectable horizontal shear across the surface fronts. Recent measurements with parachute drogues at 80 m on either side of the boundary have shown eastward flow of up to 25 cm·s^{-1}, but again no detectable shear across the front whose scale was 500 m (Horne 1978). This observation is consistent geostrophically with the hydrographic data which suggests that the baroclinic shear ΔU across the front is a factor of four smaller than the barotropic unsheared component represented by the surface current, U, i.e.

$$\frac{\Delta U}{U} = \frac{g(\Delta D)_y}{g\zeta_y} = \frac{\frac{\Delta\rho}{\rho} g\eta_y}{U} = \frac{6}{25} = 0.25,$$

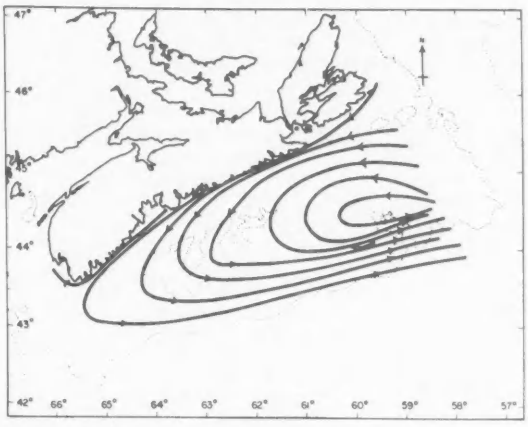

FIG. 5. Net surface circulation during August–November 1954 based on drift bottle returns (from Trites and Banks 1958).

where $\eta_y \simeq 2 \times 10^{-3}$ is the frontal slope and $\Delta\rho = 0.3 \times 10^{-3}$ g·cm^{-3}. Since the strongest horizontal shears are expected at these very narrow frontal features, it may be concluded the barotropic component of the mean flow dominates in the upper 100 m of the slope water and together with the intense variability obscures the baroclinic component.

Over the shelf, the net southwest transport near the coast appears to be well established by water mass analysis (Hachey 1938) and geostrophic calculation (Sandstrom 1919). On a broad scale, Sutcliffe et al. (1976) have recently found a correlation between St. Lawrence River discharge and ocean temperatures, which implies a pathway of influence from the Gulf of St. Lawrence to the Gulf of Maine. Net drift velocities inferred from the progression of surface temperature anomalies along the coast are found to be 6–10 cm·s^{-1} to the southwest. Measurements of longshore drift of oil particles by Forrester (1971) gave a similar result 8 cm·s^{-1}. Over the outer portion of the shelf, Trites and Banks (1958) have inferred eastward surface currents on the basis of drift bottle returns so that the net circulation over the shelf would consist of a large cyclonic gyre, as shown in Fig. 5. This result is consistent with the expected surface wind drift, since during the experiment the 15-d average wind was consistently from the southwest quadrant. More generally, drift bottle results of Bumpus and Lauzier (1965) indicate that this pattern exists throughout the year at the surface.

Recently, K. F. Drinkwater and W. H. Sutcliffe (personal communication, P.O. Box 1006, Dartmouth, N.S.) have summarized the measurements of surface drift on the shelf and compared them with geostrophic calculations from the Halifax section. They found reasonable agreement with the nearshore surface currents (particularly in winter) when the level of no motion was taken at the bottom (or 100 m in Emerald Basin). The monthly geostrophic transport along the shelf was found to be concentrated within 100 km of the coast and to reach a maximum in January (0.5×10^6 m^3·s^{-1}) and a minimum in August (0.08×10^6 m^3·s^{-1}) with an annual mean of 0.3×10^6 m^3·s^{-1}, i.e. a factor of 3 smaller than McLellan's (1957) estimate. Although these results would indicate seasonal variation, a close examination of the geostrophic data indicates that differences up to 80% of the total annual transport may occur between sections taken only 2 wk apart. Hence the dominant times scales may be much shorter than seasonal. An attempt was also made to compare the depth integrated currents from the 1967–68 mooring data with the geostrophic results. Although one favorable comparison resulted near the shore, the mean currents were quite small (<3.0 cm·s^{-1}) and of opposite signs over the outer part of the shelf. Thus the balance between Coriolis and pressure forces presumed in the geostrophic method is not valid everywhere on the shelf and may not be dominant anywhere.

Variability

Apart from the annual cycle the major sources of variability in the shelf and slope waters are (1) tides, (2) winds, and (3) the deep ocean. Our understanding of the relative importance of these forces is based primarily on the results of the 1967–68 mooring program. With sufficiently long records, it should be possible to use spectral analysis to separate the responses to each force in frequency space with tidal motions at the high end. Unfortunately, there are at present only a few records with which to examine the lowest frequency response to the deep ocean (Petrie and Smith 1977), and spectral estimates in those bands are not very stable. Therefore, these motions will be excluded from further consideration and only the wind-driven and tidal motions will be discussed in this section.

TIDAL MOTIONS

The dominant tidal component on the Scotian Shelf and Slope is the semidiurnal M_2 constituent. Using current meter data from 1967–68, Petrie (1974, 1975) has demonstrated that the M_2 is mainly barotropic over the outer shelf in all seasons. He supports this conclusion by showing that (1) the tidal currents and phases are nearly constant with depth and time except near the bottom where amplitudes are attenuated by friction, (2) the vertical and horizontal coherence of currents is high at M_2, and (3) the measured currents and surface elevation gradients satisfy the shallow water equations. At Stations 3, 6, and 7 (Fig. 1), the tidal amplitudes at a given depth varied by <10% throughout the mooring period, while vertical variations excluding the bottom sensor were limited to 2% despite marked changes in stratification. Vertical coherences over the water column were in the range 0.91 ± 0.04 with phase differences of $15 \pm 10°$, while horizontal coherences averaged 0.90 (95% confidence is at 0.33). Petrie also showed from the data that the amplitude of the onshore component of the tidal flow

TABLE 1. a, Comparison of measured and predicted onshore amplitude of the M_2 tidal stream on the Scotian Shelf. Theoretical Reynolds stress divergence, $-\frac{1}{2}(\overline{v'_0{}^2})_y$ corresponds to predicted amplitude. b, Gradients of M_2 tidal elevation based on current meter and tide gauge data.

a. Stn.	Bottom (m)	Distance from shore (km)	Measured onshore amplitude ($cm \cdot s^{-1}$) 20 m	50 m	bottom	Model amplitude $v'_0(y)$ ($cm \cdot s^{-1}$)	Shelf-wide Reynolds stress divergence ($\times 10^5$ $cm \cdot s^{-2}$)
3	95	138	16.4	16.5	11.9	12.8	1.12
6	135	121		11.5	10.9	7.7	0.49
7	125	161		13.9	10.2	11.0	0.75

b.	$\zeta_y(\times 10^6)$	Phase	$\zeta_x(\times 10^6)$	Phase
Current meters (Stn. 3, 6, 7)	1.25	235	0.83	100
Coast	0.22	223	0.31	133
Coast/Sable Is.	0.65	185		
Avg (C.M. + coast)	0.73	230		

v_0' could be closely predicted using the shore elevation ζ_0' (60.8 cm) and the local depth, h,

$$v_0' = \frac{2\pi\zeta_0'(L - y)}{\tau h},$$

where L is the shelf width and τ is the tidal period (Table 1a). This simple formula, based on the large barotropic wavelength of M_2 and continuity principles, may also be used to estimate divergences of the mean momentum flux, $\frac{1}{2}(\overline{v_0'^2})_y$, associated with onshore tidal streams on the shelf. Values of these shelf-wide Reynolds stress gradients calculated for stations on the outer banks (Table 1a) are of order 10^{-5} $cm \cdot s^{-2}$ and reach a maximum at Station 3.

In addition, Petrie was able to achieve a crude momentum balance from current meter and elevation data. Using the shallow water momentum equations

$$u'_t - fv' = -g(\zeta'_x - \lambda\zeta^0_x),$$
$$v'_t + fu' = -g(\zeta'_y - \lambda\zeta^0_y),$$

where f is the inertial frequency, λ is a constant, and ζ^0 is the equilibrium constituent, he solved for the longshore velocity at the coast (where $v' = 0$)

$$u'_{\text{coast}} = -g \int (\zeta'_x - \lambda\zeta^0_x)_{\text{coast}}\, dt,$$

and computed the offshore elevation gradient

$$(\zeta_y)_{\text{coast}} = -\frac{fu'}{g} + \lambda\zeta^0_y.$$

He then compared the average of the coastal and current-meter-derived gradients with the mean gradient computed between the coast and Sable Island (Table 1b). The agreement in both amplitude and phase between these independent estimates strengthens the conclusion that the M_2 tide is barotropic on the shelf. Discrepancies may be due, in part, to a small baroclinic component ($\sim$1 $cm \cdot s^{-1}$ at Station 3) or to frictional effects, which are estimated at roughly 5% of the leading terms in the momentum equations based on a quadratic stress law.

Over the continental slope, the M_2 tidal regime was found to be quite different. Large baroclinic tidal flows were found at Stations 4 and 5 in the 1967–68 data and again at Stations 1A and 2A in 1973. The largest amplitudes were found near the surface (20 m) at Station 4 and appeared to be phase-locked to the surface tide. Furthermore, pairs of temperature records separated by 20 m in the 1973 experiment indicated strong oscillations in vertical velocity and isotherm displacement. It was apparent that the internal tide was somehow being generated locally over the Scotian Slope.

According to Baines' (1973, 1975) two-dimensional model, an internal wave of tidal frequency may be generated by the interaction between the surface tide and a sloping bottom. Energy emanates from the generation site along characteristics of the internal wave equation whose slope is given by

$$c = \left(\frac{\omega^2 - f^2}{N^2 - \omega^2}\right)^{\frac{1}{2}},$$

where f, N, and ω are the inertial, Brunt Väisälä, and tidal frequencies, respectively. The most efficient generation occurs where the topography is convex upward and $c = s$, where s is the local bottom slope. Furthermore, where $\begin{Bmatrix} c > s \\ c < s \end{Bmatrix}$ the wave energy will be $\begin{Bmatrix} \text{transmitted} \\ \text{reflected} \end{Bmatrix}$ by the bottom. Figure 6 illustrates topographic profiles on the Scotian Slope intersecting Stations 4 and 5 and 1A and 2A (Petrie 1975). Each profile is characterized by critical zones ($c = s$) near 200 and 600 m, separating a reflective regime from two transmissive regimes on the shelf and outer slope. Characteristics based on average density profiles for the region are drawn from each critical zone to theoretically depict beams of energy. Petrie (1975) demonstrated that the intersections of these beams of energy with the moorings were consistent with the observed structure of the M_2 tide. In particular, the near-surface and near-bottom meters at Station 4 showed higher amplitudes than the one at middepth, while the

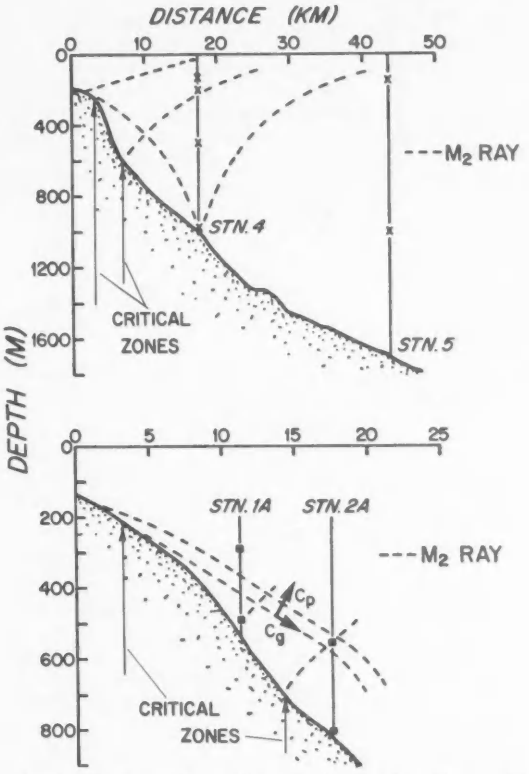

FIG. 6. Topographic profiles on the Scotian Slope with positions of moorings 4, 5, 1A, and 2A. Arrows mark critical zones ($c = s$) on the slope near 200 and 600 m (from Petrie 1975).

TABLE 2. Phase relationships for 490- and 555-m sensors in 1973 experiment.

(Coherence, phase)	Theory	Mooring 1A 490 m	Mooring 2A 555 m
U–V	(1, −90)	(0.72, −87)	(0.90, −78)
V–T	(1, −90)	(0.87, −90)	(0.82, −102)
U–T	(1, 180)	(0.71, −168)	(0.80, 177)
Amplitude ratio $\lvert U\rvert^2/\lvert V\rvert^2$	$\frac{f^2}{\omega^2} = 0.5$	0.42	0.69

sensors at 490 and 555 m on moorings 1A and 2A were the most energetic.

In an attempt to confirm the generation of internal tides near the shelf break, Petrie first tested the measured baroclinic M_2 component against the radiation condition, which requires that the group velocity have a component in the offshore direction and that the phase propagate in a direction normal to the rays. Using the 1973 records for the offshore component, he found high coherence at 0.72 (95% confidence limit is 0.45) with 490 leading

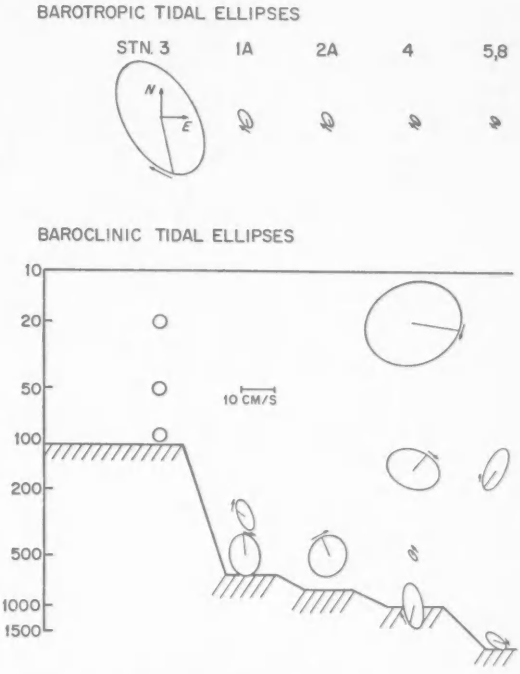

FIG. 7. A summary of M_2 barotropic and baroclinic tidal ellipses on the Scotian Shelf and Slope (from Petrie 1975).

555 m by 32 ± 14° as expected. He showed that the phase relationships and amplitude ratios at these two sensors were consistent with internal waves as shown in Table 2. However, using the observed phase lags between V_{490} and V_{555}, Petrie derived horizontal and vertical wavelengths over the slope of 42 and 1.3 km, respectively. Because the vertical scale exceeds the depth by a factor of 2, boundary effects not accounted for in Baines' theory may be important. As a check he then recomputed the vertical scale, $2\pi/m$, using the measured offshore and vertical velocities and the horizontal wave number $l = 2\pi/42$ km in the formula

$$V = -\frac{l}{m}\left(\frac{N^2 - \omega^2}{\omega^2 - f^2}\right) W.$$

This time the vertical scale was estimated at 556 m, roughly equal to the local depth. Thus the two-dimensional internal wave model is not fully satisfactory for interpreting the baroclinic tidal data.

A summary of the M_2 barotropic and baroclinic tidal ellipses is presented in Fig. 7. If the predominance of the baroclinic component over the slope is caused by local generation, then its absence over the outer portion of the shelf (Station 3) implies that the shoreward component of the internal tide is dissipated within approximately one wavelength of the shelf break. LeBlond (1966) has shown that the most likely mechanism for this process is bottom friction, not internal damping. Using scales of

TABLE 3. Nonlinear interaction terms for baroclinic M_2 tide at Station 1A: Experimental values vs. two-dimensional, internal wave on a flat bottom.

Depth	U (cm·s^{-1})	θ_x (°)	V (cm·s^{-1})	θ_y (°)	W (cm·s^{-1})	θ_z (°)	A_0 (×10^5 cm·s^{-2})	A_2 (×10^5 cm·s^{-2})	B_0 (×10^5 cm·s^{-2})	B_2 (×10^5 cm·s^{-2})
					Experimental Values					
290	2.9	170	4.6	56						
490	4.0	76	6.2	337	0.38[a]	130	−0.123	4.85	6.45	6.64
					0.33[b]	277	2.38	4.83	−5.42	5.76
					Theoretical Values (Mode 1)					
290	2.9	90	4.6	0						
490	4.0	90	6.2	0	0.36	90	−1.0	1.0	0	1.4
					(Mode 2)					
290	2.9	270	4.6	180						
460	4.0	90	6.2	0	0.36	90	−6.2	6.2	0	9.7

[a]2-h averages used for $\overline{T}_z$ in $W = T_t/\overline{T}_z$.
[b]3-h averages used for $\overline{T}_z$ in $W = T_t/\overline{T}_z$.

14 km and 100 m for the shelf, Petrie (1975) has also shown that a vertical eddy viscosity of 30 cm^2·s^{-1} is sufficient to dissipate the internal tide within 15 km of the generation site. This does not imply a transfer of momentum to the mean flow, since the tidal momentum is yielded to the boundary by the oscillating bottom stress. However, second-order bottom currents may be induced by the nonlinear advection terms (Hunt and Johns 1963).

Evidence for nonlinear interaction of the M_2 tide was also found by Petrie (1975), who noted strong 6-h oscillations in the current and temperature fields on the slope. By comparing estimates of the advection and acceleration terms, he concluded that only the stresses on horizontal planes (e.g. $\overline{w'v'}_z$) were important in generating the shallow water tides. Using expressions of the form

$$v'_{M_4} = \frac{(w'v'_z)_{M_2}}{\omega_{M_4}},$$

where ω_{M_4} is the harmonic frequency, he was able to derive amplitudes for the shallow water constituents, M_4 and MS_4, which were somewhat smaller than the measured values, but of the right order. In addition to harmonic forcing, the nonlinear interaction of M_2 results in a steady, second-order momentum flux. Interest here will be focused on this rectifying term.

Consider Petrie's (1975) data, from Station 1A, of the form

$$u'_{290} = U_2 \cos(\omega_{M_2} t - \theta_{2x}) \quad v'_{290} = V_2 \cos(\omega_{M_2} t - \theta_{2y}),$$

$$u'_{490} = U_4 \cos(\omega_{M_2} t - \theta_{4x}) \quad v'_{490} = V_4 \cos(\omega_{M_2} t - \theta_{4y}),$$

$$w'_{490} = W_4 \cos(\omega_{M_2} t - \theta_{4z}).$$

Then the divergence of the longshore Reynolds stress over the 200-m range is of the form

$$w'u'_z = A_0 + A_2 \cos(2\phi_x - \alpha_x),$$

where

$$\phi_x = \omega_{M_2} t - \theta_{4x}.$$

$$A_0 = \frac{W_4}{2\Delta z}\left[\cos\gamma_x(U_2\cos\delta_x - U_4) + U_2 \sin\gamma_x \sin\delta_x\right]$$

$$A_2 = \frac{W_4}{2\Delta z}\{[\cos\gamma_x(U_2\cos\delta_x - U_4) - U_2\sin\gamma_x\sin\delta_x]^2 + [\sin\gamma_x(U_2\cos\delta_x - U_4) + U_2\sin\delta_x\cos\gamma_x]^2\}^{\frac{1}{2}}$$

and $\Delta z = 200$ m, $\gamma_x = \theta_{4z} - \theta_{4x}$, $\delta_x = \theta_{2x} - \theta_{4x}$. A similar form results for the term, $w'v'_z = B_0 + B_2 \cos(2\phi_y - \alpha_y)$. The appropriate amplitudes and phases for the horizontal components of the baroclinic tidal streams may be applied directly from Petrie's data to compute the amplitudes A_0, A_2, B_0, B_2. However, an ambiguity arises in assigning values, particularly phase, for the vertical velocity, since conflicting values are found when different averaging periods are used to determine the vertical temperature gradient in the formula, $w' = T_t/\overline{T}_z$, from which the vertical velocity is computed. Therefore, Table 3 gives results for the nonlinear interaction amplitudes at 490 m based on two different experimental values for W_4 and θ_{4z}. For the sake of comparison, theoretical values for the nonlinear amplitudes in a flat-bottomed, two-dimensional internal wave propagating seaward are also included in Table 3. Petrie's estimates of vertical wavelength (1.3 and 0.56 km) correspond to modes 1 and 2 so both results are given. Using measured amplitudes and theoretical phase relationships, the mode 2 values represent 12% of the linear acceleration terms, i.e.

$$\frac{|w'u'_z|}{|u'_t|} = \frac{|w'v'_z|}{|v'_t|} = 0.12.$$

The purely experimental values, though somewhat inconsistent, give magnitudes that intermediate between those of the two theoretical modes.

Petrie's (1974) attempts to model the diurnal tidal flows on the shelf and slope were less successful than with

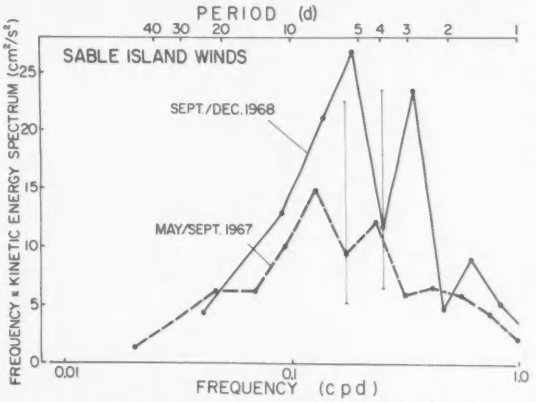
PERIOD (d)
40 30 20 10 5 4 3 2 1
SABLE ISLAND WINDS
SEPT./DEC. 1968
MAY/SEPT. 1967
FREQUENCY × KINETIC ENERGY SPECTRUM (cm²/s²)
0 5 10 15 20 25
0.01 0.1 1.0
FREQUENCY (c p d)

FIG. 8. Kinetic energy spectra for Sable Island winds in two seasons (from Petrie and Smith 1977). Vertical bars denote the 95% confidence limits from Munk et al. (1959) (cpd = cycles per day).

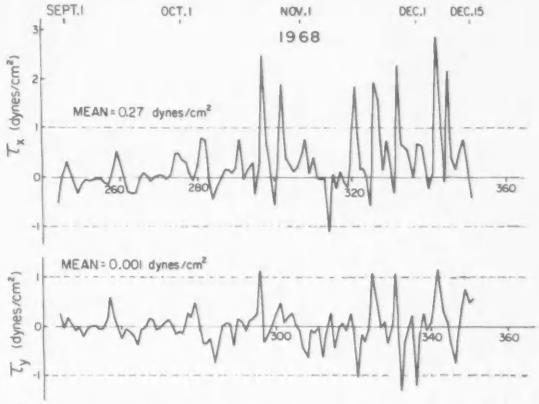

FIG. 9. Sable Island wind stress from September to December 1968 (Petrie and Smith 1977). Wind stress calculated after Smith and Banke (1975) (1 dyne = 10 μN).

the M_2 component. A step-shelf model similar to that of Munk et al. (1970) which uses coastal elevation data to predict offshore currents produced amplitudes ($\sim$1 cm·s^{-1}) 6 times smaller than those observed. Furthermore an observed baroclinic component in the current meter records on the outer banks could not be accounted for by a trapped internal mode at the shelf break because the theoretical trapping scale (3 km) was too small. An attempt to predict elevation and phase at Sable Island from the current meter data was also unsuccessful apparently due to the presence of an amphidromic point east of Sable Island (Dohler 1964). However, it was found that the step-shelf model could be forced to produce current amplitudes in the observed range by relaxing the radiation condition and imposing the longshore scale observed at the coast. This suggests the possibility of offshore driving of the diurnal tides on the shelf by a forced mode of the Atlantic itself.

Atmospheric Effects

Transient wind events over the Scotian Shelf produce two distinct types of variability: (1) strong inertial currents and (2) forced oscillations at atmospheric time scales. In this section each response will be examined after a brief description of the atmospheric forcing function.

Winds measured at Sable Island have been shown to be representative of conditions over the shelf and slope at least up to frequencies of 1 cycle/d (Petrie and Smith 1977). Figure 8 shows two spectra of the kinetic energy in the winds at Sable Island for two seasons: the summer of 1967 and the autumn of 1968. Nearly all the energy in the wind field is contained between 2- and 10-d periods for both seasons; however, the total variance during the September–December period is nearly twice as great as in May–September. In Figure 9 wind stress components to

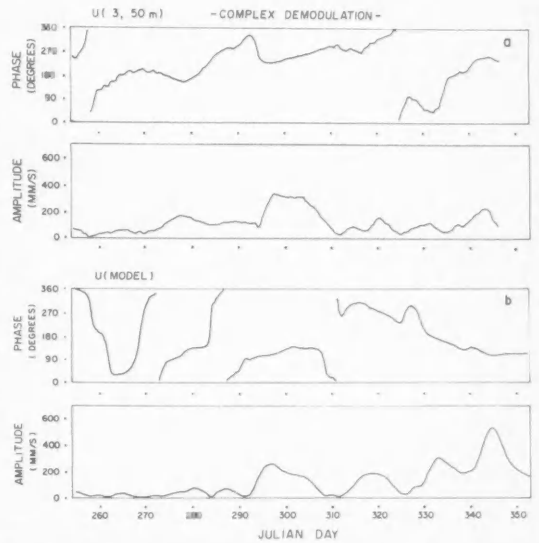

FIG. 10. a, Station 3 eastward current at 50 m, complex demodulated at inertial frequency (1.38 cpd), during September–December 1968. b, Currents from wind-driven, variable depth mixed layer model for period September–December 1968, complex demodulated at inertial frequency (1.38 cpd).

the east (τ_x) and north (τ_y) are plotted for the fall of 1968. The mean stress is almost due east at 0.27 dyne·cm^{-2}. However, the striking feature of the stress is its variability. After a relatively quiet September, there is a sharp transition (near day 295) to a succession of strong winter storms characterized by bursts of 1–3 dynes·cm^{-2} on time scales of several days. Therefore the most intense oceanic response is expected from mid-October to mid-December, unless a shelf mode resonance occurs in another season.

Inertial motions — The transient character of inertial currents over the shelf is most easily seen by complex demodulation of the current records at the inertial frequency (1.38 cycles/d). Figure 10a shows the demodulated amplitude and phase for inertial motion in the eastward current at Station 3, 50 m, during September–December 1968. Each peak in the amplitude may be directly identified with a pulse in the wind stress of Fig. 9 (although the converse is not true). For example, the sharp rise near day 295 corresponds to the transition to the winter storm period. The demodulated amplitude corresponds to magnitude of the Fourier component at inertial frequency averaged over 5 d. Hence the inertial motion in the U component alone is over 30 $cm \cdot s^{-1}$ for 10 d following day 295. The constancy of the phase function when the amplitude is high indicates that the frequency of the response is very nearly inertial. Notice also that the response to individual wind stress pulses usually decays within several days. These results are similar to those of Pollard and Millard (1970), who modeled the response of the mixed layer in the open ocean to local winds and successfully compared their results with current measurements at Site D on the New England continental rise. They noted that rapid changes in wind stress magnitude or direction could create and destroy inertial motion. A similar model has been constructed to analyze inertial motion on the Scotian Shelf.

In the initial attempt, the surface wind stress was calculated from hourly wind measurements taken at Sable Island using the drag coefficient model of Smith and Banke (1975) and used to drive the Pollard and Millard model for the September–December period. A mixed-layer depth of 50 m and a frictional coefficient of $2.5 \times 10^{-6} \cdot s^{-1}$ were assumed. The model was reasonably successful in duplicating the variations but not the amplitudes of the inertial currents. The current magnitudes were too low in September and too high in December, agreeing in between. An examination of hydrographic sections (de la Ronde 1972) taken during the mooring period indicated that the mixed-layer depth increased from about 10 m in early September to a weakly stratified condition (one σ_t unit over 100 m) in December. Thus, it seemed desirable to formulate a model with a variable-depth mixed layer.

Such a model, based on the Pollard et al. (1973) formulation of the deepening of the mixed layer, was incorporated into the inertial wave study. The model is based on a Froude number criterion: when the Froude number equaled or exceeded one, the layer depth was incremented by 5 m and the momentum and density were conserved. The initial density profile was linear and based on hydrographic data from de la Ronde (1972) and the starting layer depth was taken as 10 m. Since the uppermost current meter was well below the mixed layer at least at the start of the record, an additional assumption that the inertial energy generated in this layer would propagate undiminished to 50 m was necessary. In comparing the theoretical results (Fig. 10b) to the actual data (Fig. 10a), most of the prominent features are reproduced.

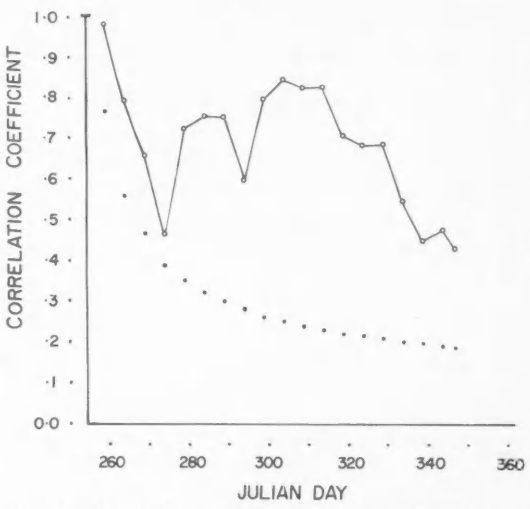

FIG. 11. Correlation coefficient for eastward currents at inertial frequency from model and data as a function of time. Dots indicate the 0.01 significance level (Fisher and Yates 1970).

Although the amplitudes of the variable-depth model agreed with the data better than those from the constant-depth version, they are still underestimated at the start of the record and overestimated at the end. Phase agreement (note that there is an arbitrariness in the absolute value of the phase) is fairly good for the first two-thirds of the record, especially days 295–309, but the phases show opposite trends for the last third of the record. The cumulative correlation coefficient for the eastward velocity components, model versus data, is shown in Fig. 11 as a function of time. During the first 75 d the correlation is around the 0.70 level, indicating good agreement. However, it drops precipitously to 0.43 during the last 18 d. At all times, though, the coefficient exceeds the 0.01 level of significance. By the end of the data series the model mixed-layer depth had increased to 65 m.

Despite its simplicity, the variable-depth model has successfully reproduced the inertial wave amplitudes and variations at 50 m on the Scotian Shelf. The novel feature of this model is that the forcing term, and hence the response, are inversely proportional to the mixed-layer depth, $h(t)$, whose growth is determined by the stability of the interface at the base of the layer. The model's behavior appears to be borne out by the data, but the assumption that the inertial energy reaches the 50 m instrument undiminished when it lies outside the mixed layer is difficult to justify. Indeed, Pollard's (1970) approximate solution for long, wind-driven inertial waves in a stratified fluid predicts that for times

$$t \ll \frac{f^2\lambda^2}{2N^2H^2} \simeq 100 \text{ d},$$

where λ ($\sim$500 km) is the forcing wavelength, N/f ($\sim$100) is the ratio of Brunt Väisälä and inertial frequencies, and H ($\sim$100 m) is the total depth, the response below the forced layer is independent of h. But this result, in turn, depends on Pollard's idealization for mixed-layer forcing, particularly the penetration depth for momentum. Obviously, more detailed observations leading to a better model for mixed-layer dynamics are required before the mechanism(s) for wind forcing of inertial currents is understood.

The question of horizontal and vertical scales of inertial motions on the Scotian Shelf was addressed by Warner (1970) in a detailed cross-spectral analysis of data from the 1967 experiment (Grant and Reiniger 1970). He found that bursts of inertial energy with amplitudes of 30–40 cm·s^{-1} were common at depths of 20–50 m and occasionally led to high horizontal coherences of over distances of order 50 km. The results, particularly for phases, were highly erratic, however, so estimates of horizontal wavelength were not attempted. At Stations 2 and 3 some high vertical coherences accompanied by a phase shift of 180° were also found in the surface layer between 20 and 50 m. These observations are confirmed by the coherence and phase (0.74, −174°) at inertial frequency between the clockwise components (see Gonella 1972) of the vector time series at Station 3, 20, and 50 m, in September–October 1968. The horizontal coherence and phase between Stations 2 and 3 at 50 m are (0.78, 34°). [For these statistics the 95% confidence level for coherence is 0.49 (Benignus 1969) and the bounds on the phase are roughly ±8° (Jenkins and Watts 1968).] The horizontal phase difference implies a wavelength of $\sim$500 km, whereas the vertical phase variation means that there is a strong vertical shear in the inertial currents. The kinetic energies in the inertial frequency band for this period are equivalent to inertial current of 8.3 and 10.5 cm at 20 and 50 m, respectively, so that despite the phase change the amplitude is relatively uniform. Unfortunately, the overlapping portion of these records, days 251–292, 1968, does not include the most energetic inertial response at the 50-m level (Fig. 10), so the relevance of the scale estimates is questionable. The inertial amplitude for the 50-m record (September–December) is 20.5 cm·s^{-1}, reflecting the input of inertial energy by wind.

Records from deeper instruments indicate that the inertial energy is significantly attenuated below 50 m. Propagation rates deduced from amplitude variations in the complex demodulated signals are highly variable, but on at least one occasion at Station 2 in 1967, a pulse of inertial energy was traced from the surface (20–50 m) to 250 m in $\sim$3 d while suffering a threefold reduction in amplitude (Warner 1970). Thus transient inertial oscillations are felt to some extent in the deepest waters of the shelf, but simple models cannot explain the mechanism for vertical propagation of inertial energy.

Finally, with regard to the mean dynamical balance, some generalizations may be made about the Reynolds stresses associated with inertial currents. First, since the horizontal components are very nearly in quadrature, the term $\overline{u'v'}$ may be eliminated. Second, the stresses on horizontal planes (e.g. $\overline{u'w'}$) are confined primarily to the forced layer, since the motion is basically horizontal and is strongly attenuated in the deeper shelf waters. The most important inertial stresses appear to be the squared terms $\overline{u'^2}$ and $\overline{v'^2}$ but because of the large horizontal scales associated with the motion ($\sim$500 km), the maximum divergences of these stresses should occur for the onshore component, $\overline{v'^2}$, which must vanish at the coast. The magnitude of $\overline{v'^2}$ in the inertial band may be comparable to that in the semidiurnal band near the surface during stormy seasons (see Fig. 12). The Reynolds stress divergence for the onshore flow will be estimated in the next section.

Fluctuations at synoptic atmospheric frequencies — Current oscillations at periods of 2–10 d are also forced by atmospheric winds. This fact is evident in the comparison of current spectra from the fall of 1968 (Fig. 12a, b) and summer of 1967 (Fig. 12c). Kinetic energy in the atmospheric and inertial frequency bands are considerably different for the two periods, whereas tidal energies are comparable. Furthermore individual peaks in the current amplitude have been traced directly to meteorological events during September–December 1968 (Petrie and Smith 1977). The absence of "ringing" in the wake of isolated storms indicates that the response is primarily a forced wave moving with the driving system (i.e. eastward), rather than a set of westward-propagating free modes. Notice also that since the major storm track is west to east over the shelf, shelf mode resonance is unlikely.

The cross-shelf circulation at these frequencies has been shown to be of the classical Ekman type in the nearshore environment. Hachey (1937), in examining 8 yr of data from the month of September, found a strong negative correlation between longshore (eastward) wind at Sable Island and Halifax Harbour surface temperature, which he attributed to upwelling at the coast. Similarly, Longard and Banks (1952) interpreted changes in bottom temperature at Sambro light vessel (18.5 km offshore at Halifax) to wind-driven Ekman circulation. More recently, Sandstrom (personal communication, P.O. Box 1006, Dartmouth, N.S.) has found significant coherence over a broad range of frequencies between adjusted sea level at Halifax and 2 yr of wind data at CFB Shearwater. For periods longer than 2 d, the sea level fluctuations are in phase with those of the wind indicating once again a direct response to the forcing.

Longshore winds over the outer banks on the shelf are thought to produce intrusions of slope water consistent with Ekman dynamics. Two cases of intrusions following a period of strong winds have been hydrographically documented by Hachey (1953) and McLellan et al. (1953). A similar phenomenon occurred in the fall of 1968 (Fig. 13). The two salinity sections taken in October and December of that year show that during that stormy season when the mean wind stress was to the east (see

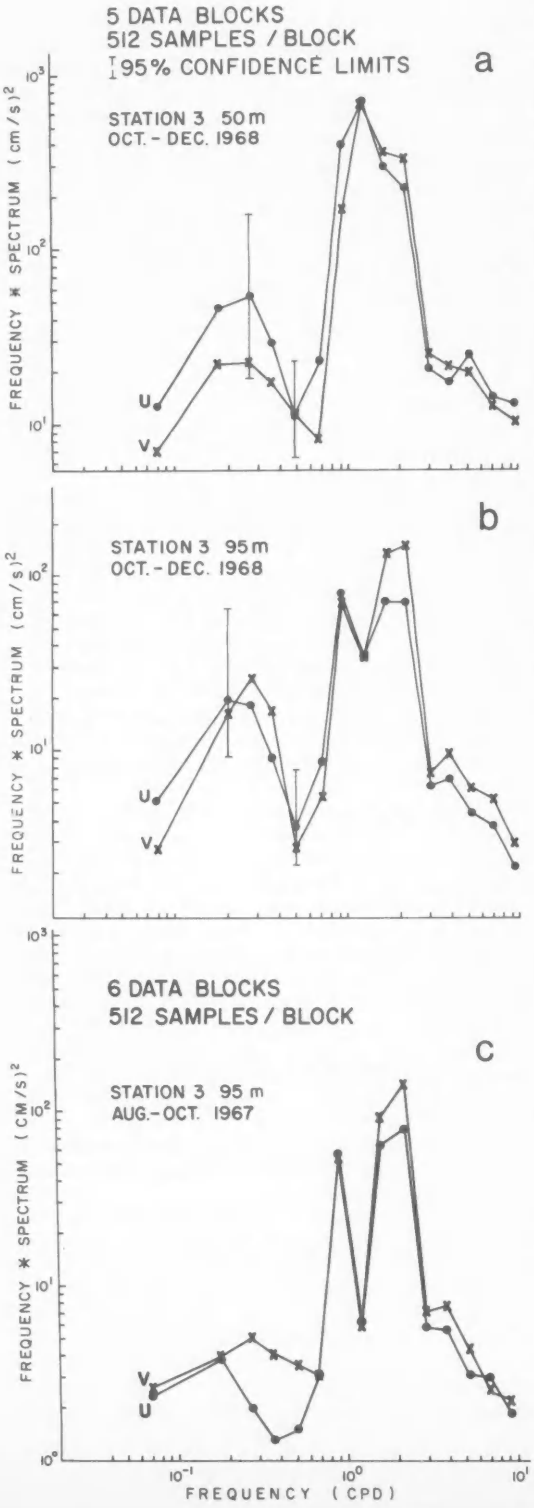

FIG. 12. Frequency times spectra for Station 3 currents in different seasons (Petrie and Smith 1977). a, 50 m, October–December 1968; b, 95 m, October–December 1968; c, 95 m, August–October 1968. Vertical bars denote the 95% confidence limits from Munk et al. (1959).

Fig. 9) a large volume of slope water ($S \geq 35‰$) penetrated onto the shelf and into Emerald Basin. Current records from that time measure a net onshore component at Station 3, 50 m, that is well correlated with eastward wind stress and constitutes the return flow required to compensate for the inferred offshore Ekman drift at the surface (Petrie and Smith 1977). Longshore advection of this deep water may be ruled out for two reasons: (a) there is no upstream source with this high salinity, and (b) rough topography prevents communication between the deep waters of the northeastern and southwestern portions of the shelf (Hachey 1942).

To assess the importance of wind-driven and tidal Reynolds stresses to the mean momentum balance, Fig. 14 presents the cospectra of horizontal current components at Stations 2 and 3 for the fall period, September–December 1968. For convenience the current is resolved into eastward (longshore), U, and northward (onshore), V, components, but the sign and order of magnitude of the momentum flux in the various frequency bands are unchanged for rotations up to 30° north of east, which represents the ambiguity in orientation of the Nova Scotian coastline and shelf break. The shapes of these curves are similar for all depths at both stations. In the wind-driven (W) and diurnal (D) bands, there are distinct onshore momentum fluxes, while in the semidiurnal band (SD), the flux is offshore. As expected, the momentum flux in the inertial band (F) is consistently near zero. These data will be used to compute Reynolds stress divergences for the mean momentum balance in the next section.

Mean Momentum Balance

Now we consider the question of the mean momentum balance on the Scotian Shelf. Since the Nova Scotian coastline is long and relatively straight, equations (1)–(3) in the first section may be simplified by neglecting longshore variations in the velocity and density fields and integrating vertically to obtain the barotropic mean momentum equations:

$$(4)\quad 0 = f\langle V\rangle - g\zeta_x + \frac{\tau_w{}^x - \tau_b{}^x}{\rho H} - \langle\overline{u'v'}\rangle_y - [\langle\overline{u'v'}\rangle - (\overline{u'v'})_{-H}]\frac{H_y}{H},$$

$$(5)\quad 0 = -f\langle U\rangle - g(\zeta + D)_y + \frac{\tau_w{}^y - \tau_b{}^y}{\rho H} - \langle\overline{v'^2}\rangle_y - [\langle\overline{v'^2}\rangle - (\overline{v'^2})_{-H}]\frac{H_y}{H},$$

where $H(y)$ is the depth, $\langle\langle(\,)\rangle\rangle = (\zeta + H)^{-1}\int_{-H}^{\zeta}(\,)\,dz \simeq H^{-1}\int_{H}^{0}(\,)\,dz$, and the following boundary conditions have been applied:

$$(\tau_w{}^x, \tau_w{}^y) = -\rho(\overline{u'w'}, \overline{v'w'}) \quad \text{at } z = \zeta \simeq 0,$$
$$(\tau_b{}^x, \tau_b{}^y) = -\rho(\overline{u'w'}, \overline{v'w'}) \quad \text{at } z = -H,$$

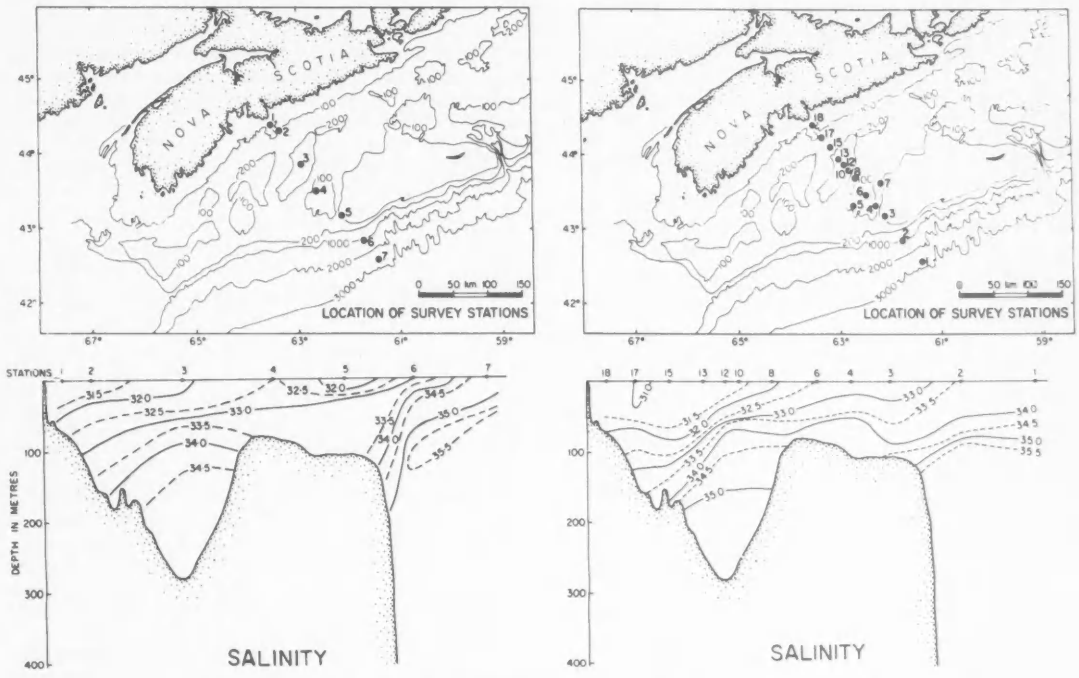

FIG. 13. Salinity contours for hydrographic sections from October 20 to 21, 1968 and December 16 to 17, 1968 (Petrie and Smith 1977).

where $(\tau_w{}^x, \tau_w{}^y)$ and $(\tau_b{}^x, \tau_b{}^y)$ are the mean wind and bottom stresses. The topographic terms arise as follows ($\zeta \ll H$):

$$H\langle(\overline{u'v'})_y\rangle \simeq \int_{-H}^{0}(\overline{u'v'})_y\,dz = \frac{d}{dy}\int_{-H}^{0}\overline{u'v'}dz - (\overline{u'v'})_{z=-H}\frac{dH}{dy}$$
$$\equiv [H\langle\overline{u'v'}\rangle]_y - (\overline{u'v'})_{-H}H_y$$
$$= H\left\{\langle\overline{u'v'}\rangle_y + [\langle\overline{u'v'}\rangle - (\overline{u'v'})_{-H}]\frac{H_y}{H}\right\}$$

and vanish if the momentum flux is vertically uniform or the bottom is flat. Following Csanady (1976), the mean longshore pressure gradient, $g\zeta_x$, is retained and enters the equations parametrically.

The current meter records for the period from September to December 1968 will be used to calculate the first-order momentum flux divergences. During this time, the mean wind stress components over the shelf were $(\tau_w{}^x, \tau_w{}^y) = (0.27, 0.001)$ dyne·cm^{-2} (see Fig. 9). Because of the dominance of the first-order variable flow, the bottom stress may be estimated simply from a linearized formula based on a quadratic stress law (Rooth 1972)

$$\frac{(\tau_b{}^x, \tau_b{}^y)}{\rho H} = \frac{3}{2H}C_D\bar{C}(U, V),$$

where C_D is the drag coefficient (2×10^{-3}) and $\bar{C}$ is the root mean square amplitude of the near-bottom first-order flow (16.0 cm·s^{-1} at Station 3, 95 m, see Fig. 12b). Using Drinkwater and Sutcliffe's (personal communication) geostrophic calculations and 1967–68 mooring data, estimates of the vertically averaged longshore component of the mean flow, $\langle U\rangle$, in the top 100 m vary from -2 to -10 cm·s^{-1} in the Nova Scotian Current (between Station 2 and the shore) to between 0.0 and -1.0 cm·s^{-1} over the outer banks, with a shelf-wide annual mean of -1.7 cm·s^{-1}. Naturally the assumption underlying the geostrophic computations is a balance between the first two terms in equation (5). This hypothesis will be confirmed if the other terms in this equation are found to be much smaller. Unfortunately, there appears to be no way to estimate a meaningful value for $\langle V\rangle$ from the current meter results, but Trites and Banks' (1958) results indicate that V may be negative at the surface.

To evaluate the Reynolds stress divergences caused by the first-order flow field, the momentum fluxes will be split into four spectral bands:

(W) wind-driven (periods: 2–10 d);
(D) diurnal (periods: 20–30 h);
(F) inertial (periods: 15–20 h);
(SD) semidiurnal (periods: 10–15 h).

Table 4 gives the contributions to the Reynolds stresses in each band based on the spectral data in Fig. 12 and 14 and the spectra at Station 2 (Petrie and Smith 1977). For each mooring, the Reynolds stress quoted for the bottom ($z = -H$) is that at the deeper sensor and the vertically

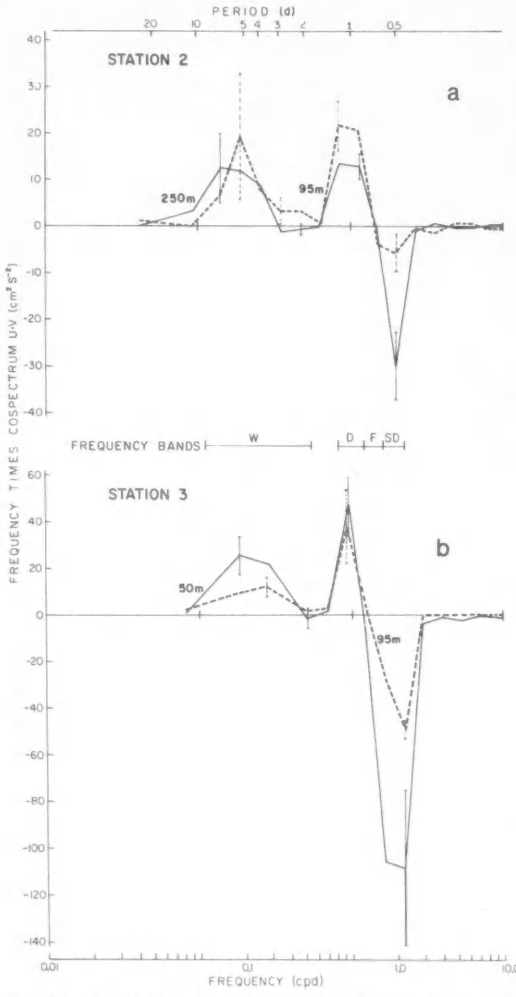

FIG. 14. Frequency times cospectrum of eastward (U) and northward (V) velocity components at Stations 2 and 3, September–December 1968. a, Station 2 at 95 and 250 m ($K = 4$); b, Station 3 at 50 and 95 m ($K = 5$). Spectral analysis is performed on K nonoverlapping blocks of data. Error bars represent standard error of block mean.

integrated stress is simply the average of the values at the two levels. Note that this procedure probably underestimates the actual inertial stresses at Station 2 because there were no records from the surface layer. The largest first-order stresses were found in the onshore spectrum at Station 3 in the inertial and semidiurnal bands. The equivalent amplitude for the barotropic semidiurnal tidal stream, $(2\langle\overline{v'^2}\rangle)^{\frac{1}{2}}$, is 16.4 cm·s^{-1} consistent with Petrie (1975) (see Table 1a). The stresses in the wind-driven bands are typically factors of 3–5 smaller than in the inertial or semidiurnal bands. Since the cospectral components in the inertial band are at the noise level, they will not be further considered.

TABLE 4. Reynolds stresses in various frequency bands at Stations 2 and 3 for September–December 1968. For spectral analysis the records were divided into five blocks. Error bounds on the spectral and cospectral estimates are taken as the standard error of the mean based on block statistics.

Spectral band (depths)	$\langle\overline{u'v'}\rangle$ (cm^2·s^{-2})	$(\overline{u'v'})_{-H}$ (cm^2·s^{-2})	$\langle\overline{v'^2}\rangle$ (cm^2·s^{-2})	$(\overline{v'^2})_{-H}$ (cm^2·s^{-2})
Wind-driven (W)				
Stn. 2 (95, 250 m)	13±9	12±9	45±23	48±24
Stn. 3 (50, 95 m)	19±8	13±5	30±13	24±10
Diural (D)				
Stn. 2	10±2	8±2	13±3	15±4
Stn. 3	13±4	11±5	40±14	21±9
Inertial (F)				
Stn. 2	−0.7±0.6	−0.3±0.1	8±2	3±1
Stn. 3	−1±3	0.9±0.5	108±44	10±3
Semidiurnal (SD)				
Stn. 2	−11±2	−21±2	20±5	28±5
Stn. 3	−42±4	−22±2	134±26	78±13

The various contributions to the mean momentum balance on the shelf may now be computed and compiled in Table 5. Where possible the estimates have been made for the inshore and offshore regions. The pressure gradient in the longshore equation (4) is that obtained near shore in the Middle Atlantic Bight by Scott and Csanady (1976) and is considered an upper bound on that term. It is reasonable to assume that a similar force is created by the outflow from the Gulf of St. Lawrence. Offshore Reynolds stress gradients were computed by differencing the average stresses in each frequency band between Stations 2 and 3 and dividing by the separation, $\Delta_{23} = 50$ km. Inshore stresses were calculated with Station 2 data and by assuming $v' = 0$ at the coast ($\Delta_{02} = 88$ km).

These results clearly show that the Reynolds stresses acting on vertical planes are insignificant to the dynamics of the inshore zone. The primary longshore balance appears to be between the pressure gradient, $-g\zeta_x$, and the wind and bottom stresses, which act in the same direction. Another candidate to balance these boundary stresses is the Coriolis force that would arise from a weak offshore mean flow of order $\langle V\rangle = -1.0$ cm·s^{-1} (provided $\langle V\rangle_y \simeq -\langle U\rangle_x \neq 0$). The two horizontal Reynolds stress gradients are at least an order of magnitude smaller than the leading terms in the nearshore region.

The onshore balance near the coast appears to be geostrophic, thus confirming Drinkwater and Sutcliffe's use of the dynamic method to compute longshore transport. The weakness of the mean onshore component of wind stress at this particular time seems to be consistent with more recent data, indicating that this term is usually negligible. Again the Reynolds stress gradients are in-

TABLE 5. Estimated magnitude of terms in the vertically averaged mean momentum equations on the Scotian Shelf ($f = 10^{-4}\cdot s^{-1}$). Frequency bands: wind driven (W), inertial (F), diurnal (D), and semidiurnal (SD).

Alongshore equation (4)			Onshore equation (5)		
	Magnitude ($\times 10^5$ cm·s^{-2})			Magnitude ($\times 10^5$ cm·s^{-2})	
Term	Inshore (Stn. 2 to coast)	Offshore (Stn. 3 to Stn. 2)	Term	Inshore (Stn. 2 to coast)	Offshore (Stn. 3 to Stn. 2)
$f\langle V\rangle$	?	?	$-f\langle U\rangle$	20 to 100	>0 <10
$-g\zeta_x$	≤ -14.4[a]	?	$-g(\zeta + D)_y$	−20 to −100	<0 >−10
$\frac{\tau_w{}^x}{\rho H}$	2.7[b]	2.7[b]	$\frac{\tau_w{}^y}{\rho H}$	0.01[b]	0.01[b]
$-\frac{\tau_b{}^x}{\rho H}$	1–5[c]	<0.5[c]	$\frac{\tau_b{}^y}{\rho H}$	?	?
$-\langle\overline{u'v'}\rangle_y$	0.14 (W)[d] 0.11 (D) −0.13 (SD)	0.12 (W)[d] 0.05 (S) −0.62 (SD)	$-\langle\overline{v'^2}\rangle_y$	0.51 (W)[d] 0.15 (D) 0.09 (F) 0.23 (SD)	−0.29 (W)[d] 0.53 (D) 2.0 (F) 2.3 (SD)
$-[\langle\overline{u'v'}\rangle - (\overline{u'v'})_{-H}]\cdot\frac{H_y}{H}$	0.003 (W)[d] 0.03 (D) −0.11 (SD)	−0.09 (W)[d] −0.03 (D) 0.34 (SD)	$-[\langle\overline{v'^2}\rangle - (\overline{v'^2})_{-H}]\cdot\frac{H_y}{H}$	−0.04 (W)[d] −0.02 (D) 0.06 (F) −0.03 (SD)	−0.12 (W)[d] −0.32 (D) −1.69 (F) −0.95 (SD)

Data sources:

[a]Longshore pressure gradient at coast in Middle Atlantic Bight (Scott and Csanady 1976).

[b]Fig. 9.

[c]Longshore geostrophic transport calculations (K. F. Drinkwater and W. H. Sutcliffe, personal communication, P.O. Box 1006, Dartmouth, N.S.).

[d]Fig. 12, 14, and Table 4.

significant compared with the geostrophic terms. Also notice that although the inertial stresses at Station 2 may be underestimated, amplification by a factor of 2 or 3 would not alter the conclusion for the inshore zone.

In the offshore region, the situation is markedly altered, particularly in the onshore equation (5). The weakening of the longshore mean flow and growth of the Reynolds stresses over the outer banks make the horizontal divergence terms comparable to the geostrophic terms. The main contributions to the first-order stress divergences come from the semidiurnal tidal streams and the inertial terms. Although both are somewhat offset by the topographic terms, they are certainly of the same order as the first two quantities in this equation. Note that the simple predictions for $\langle\overline{v'^2}\rangle_y$ in Table 1a, based on a uniform shelf-wide divergence, underestimate the measured divergence between Stations 2 and 3 by a factor of 2. In the longshore equation, (4), the stress divergences are also enhanced while the mean bottom stress is reduced. However, the wind stress is still nearly an order of magnitude greater than the net effect of the Reynolds stresses.

The importance of the first-order momentum fluxes near the edge of the shelf is confirmed by comparing the magnitudes of the terms in Table 5 with the internal stress gradients produced by the baroclinic M_2 tide (Table 3). The rectifying terms, A_0 and B_0, are comparable to the nonlinear interactions of the barotropic tide and inertial waves on the shelf and hence may provide a significant contribution to the mean momentum balance.

Discussion

Based on data from the Scotian Shelf considered here, the following conclusions may be drawn about the mean momentum balance on the shelf:

a) Reynolds stresses on vertical planes, driven by winds and tides, are insignificant in the inshore zone (within 90 km of the coast) except possibly in the coastal boundary layer where divergences may be enhanced. The main dynamic balances in this region are between pressure gradients and wind and bottom stresses in the longshore direction or Coriolis force in the offshore direction (i.e. geostrophic).

b) Over the outer banks, the geostrophic onshore balance breaks down and enhanced tidal (particularly M_2) and inertial flows produce stress divergences comparable to the geostrophic forces. In the longshore balance, the mean wind stress remains important in the offshore zone.

c) Near the edge of the shelf, the generation of the baroclinic M_2 tide results in internal Reynolds stress divergences that are comparable to the barotropic terms on the banks. These forces are capable of producing significant mean currents plus tidal harmonics by nonlinear interaction.

These results clearly show that any consideration of the dynamics of the outer Scotian Shelf or Slope Water must take into account the first-order variable flow quantities. However, since the particular conclusions listed above are based primarily on data from a single season, September–December 1968, several qualifying remarks must be added:

i) The mean wind stress providing a balance in equation (4) drops by a factor of 3–5 in summer to make the tidal stresses relatively more important.

ii) The influence of deep-ocean forcing has been excluded because the available records were too short, but the kinetic energy spectra are red and 50-d variability may be important on the shelf, particularly over the outer banks (Petrie and Smith 1977). A long-term experiment, of which the primary goal is to analyze low-frequency motion, is presently underway at the shelf break (Smith 1978).

In addition to the dynamics of the mean flow, a model for wind-driven inertial motions in a mixed layer of variable depth has been considered. In this model the growth of the mixed layer with time was related parametrically to shear instability at the interface between the layer itself and the stratified column below, as suggested by Pollard et al. (1973). The variation in thickness of the forced layer appears to be important to the inertial energy at 50 m, Station 3, but the structure of inertial currents near the surface is inconsistent with this and other models.

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Effects of the Polychlorinated Biphenyl Aroclor® 1254 on Growth, Survival, and Bone Development in Brook Trout (*Salvelinus fontinalis*)

WILBUR L. MAUCK, PAUL M. MEHRLE, AND FOSTER L. MAYER

Columbia National Fisheries Research Laboratory, Fish and Wildlife Service, U.S. Department of Interior, Columbia, Mo. 65201, USA

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Eyed eggs of brook trout (*Salvelinus fontinalis*) were exposed to Aroclor® 1254 (0.43–13 μg/L) for 10 d before hatching and the fry for 118 d after hatching. Median hatching time, egg hatchability, and sac fry survival were not affected by Aroclor 1254. At 48 d after hatching, growth was significantly ($P < 0.05$) decreased by Aroclor 1254 concentrations ≥1.5 μg/L, but no significant differences in growth of surviving fry were observed at the end of the 118-d exposure. Mortality occurred in fry exposed to 13 μg/L within 48 d of exposure, and after 118 d of exposure significant mortality occurred in the three highest concentrations. Biochemical constituents in brook trout fry related to growth and development were affected by Aroclor 1254. Hydroxyproline and vitamin C concentrations in sac fry (38 d old) were decreased by ≥3.1 μg/L. Backbone development in fry exposed for 118 d was significantly altered. Collagen was significantly decreased in the backbone as was the phosphorous concentration, while the calcium concentration increased. Hydroxyproline concentration in collagen isolated from the backbone was also decreased. The no-effect exposure concentration on backbone composition was <0.43 μg/L. Whole body residues in fry exposed for 118 d were 40 000 to 47 000 times the concentration in water.

Key words: brook trout, *Salvelinus fontinalis*, Aroclor® 1254, polychlorinated biphenyls, PCB, growth, bone development, vitamin C, collagen

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Des embryons d'omble de fontaine (*Salvelinus fontinalis*), pourvus d'yeux, ont été exposés à l'Aroclor® 1254 (0,43 à 13 μg/L) durant les 10 jours précédant leur éclosion et 118 jours après. L'Aroclor 1254 n'a eu aucun effet sur la médiane de la date d'éclosion, sur le taux d'éclosion ainsi que sur la survie des alevins munis d'une vésicule vitelline. L'Aroclor 1254, à des concentrations de 1,5 μg/L et plus, avait ralenti sensiblement la croissance ($P > 0,05$), 48 jours après l'éclosion, mais à la fin des 118 jours, les survivants ne présentaient aucun retard de croissance appréciable. Au cours des 48 premiers jours, des alevins sont morts à une concentration de 13 μg/L, et après 118 jours, les trois plus fortes concentrations ont été notablement létales. L'Aroclor 1254 a eu des effets sur les constituants biochimiques activant le développement et la croissance. Chez les alevins pourvus d'une vésicule vitelline (de 38 jours), les concentrations de l'hydroxyproline et de la vitamine C ont diminué de <3,1 μg/L. Chez les alevins exposés à l'Aroclor 1254 durant 118 la colonne vertébrale a subi des modification sensibles. Sa teneur en collagène et en phosphore a diminué tandis que celle du calcium a augmenté. Dans le collagène notamment, la concentration d'hydroxyproline a baissé. A la concentration de <0,43 μg/L, l'Aroclor 1254 n'a eu aucun effet sur la colonne vertébrale. Après 118 jours d'exposition, les alevins en contenaient 40 000 à 7 000 fois plus que l'eau.

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POLYCHLORINATED biphenyls (PCBs) are mixtures of chlorinated biphenyl chemicals that have a multitude of industrial uses as dielectric fluids in transformers and capacitors, hydraulic fluids, and plasticizers. During the past 45 yr, about 635 million kg of PCBs were produced in the United States, of which about one third now resides in landfills, air, water, soil, and sediments (USEPA 1976a).

The environmental occurrence of PCBs in aquatic

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and terrestrial habitats and its biological impact were recently reviewed (USEPA 1976b). Various toxicological studies with aquatic organisms showed PCBs to be of relatively low acute toxicity to aquatic invertebrates and fish, to have a high propensity to accumulate in the food chain, to be more toxic to early than to late life stages of fish, and to be highly persistent. However, the chronic sublethal effects of PCBs on growth and development in fish have not been studied adequately. We therefore evaluated the influence of low concentrations of the PCB Aroclor® 1254 on growth, survival, and bone development in brook trout (*Salvelinus fontinalis*).

Materials and Methods

The study was conducted according to procedures for partial chronic tests recommended by the USEPA (1972). Brook trout eggs obtained from the Nashua (N.H.) National Fish Hatchery were exposed continuously to Aroclor 1254 in a proportional diluter system (Mount and Brungs 1967) for 10 d before hatching, and the resulting fry for 118 d. Aroclor 1254 concentrations in water were determined at 2-wk intervals by gas–liquid chromatography (Hesselberg and Johnson 1972); the measured concentrations (standard deviation in parentheses) were 0.43 (0.11), 0.69 (0.12), 1.5 (0.2), 3.1 (0.4), 6.2 (1.2), and 13 (2) μg/L. An experimental-use sample of Aroclor 1254 from Monsanto Chemical Co., St. Louis, Mo., was used throughout the study. Acetone, used as the carrier solvent, did not exceed 0.30 mL/L in any concentration. Dechlorinated municipal water from La Crosse, Wis. (pH 7.7, alkalinity 260 mg/L, and hardness 305 mg/L), was delivered to each exposure tank at 333 mL/min. The water temperature was maintained at 12°C.

Median hatching time was 8–9 d after the start of the study, and median yolk absorption was completed 38 d later. The study continued for 80 d after the median yolk absorption date.

Each glass exposure container was divided into two 24-L compartments by a glass partition. Flow-splitting chambers were mounted above each container to divide the flow equally between the two compartments. One hundred eyed brook trout eggs were placed in one compartment and 150 in the other. From the 100-egg lots we determined egg mortality and percentage hatch, sac fry mortality, and fry growth and mortality. After yolk absorption, 25 fry were randomly selected for growth determinations for the remainder of the exposure. These were fed brine shrimp for several days and then fed ad libitum a modification (Mehrle et al. 1977) of the Oregon Test Diet (National Academy of Science 1973).

The 150-egg lots were used for biochemical and residue determinations. Eight pooled samples of six 38-day-old fry each were collected after the yolk was absorbed and before they were fed, for determinations of whole body vitamin C (Hubmann et al. 1969), hydroxyproline (Woessner 1961), and protein (Lowry et al. 1951). Backbones were dissected from six to eight 118-day-old fry from each concentration except the highest, in which mortality was 100%. The vertebrae were dried at 110°C for 2 h in a forced air oven, split into two fractions, and weighted. Collagen was isolated from one fraction by the method of Flanagen and Nichols (1962). The isolated collagen was weighed and subjected to hydrolysis at 115°C in 5 mL of 6 N HCl for 16 h. Hydroxyproline was determined in a 2-mL sample. The other bone fraction was subjected to hydrolysis at 115°C in 3 mL of 6 N HCl for 16 h, and calcium in the hydrolysate was determined by atomic absorption spectrophotometry and phosphorus by the Fiske and Subbarow (1925) method. Pooled samples of fry were collected and analyzed for Aroclor 1254 concentrations (Stalling and Huckins 1971) at 7, 34, 48, and 118 d after hatching. Minimum detectable limits for Aroclor 1254 were 0.1 μg/g in fish tissue and 0.1 μg/L in water, respectively.

We analyzed growth and biochemical data by analysis of variance to determine whether significant differences existed, and used a multiple means comparison test (least significant difference) to compare treatment means. Mortality data were analyzed by binomial chi-square analysis (Snedecor 1965).

TABLE 1. Median hatch time, egg hatchability, and mortality of brook trout sac fry and fry continuously exposed to Aroclor 1254 at 12°C.

Aroclor 1254 concn (μg/L)	Median hatch time (days)	Egg hatch (%)	Sac-fry mortality (%)	Fry mortality (%) after			
				48 d	59 d	73 d	118 d
0	9	92	0	0	0	0	4
0.43	9	94	0	0	0	2	2
0.69	9	91	4	0	0	2	5
1.5	9	97	0	0	0	0	5
3.1	9	94	4	0	0	10	21[a]
6.2	8	90	4	0	8	50[a]	50[a]
13	8	88	0	68[a]	96[a]	96[a]	100[a]

[a]Significantly different from the control ($P < 0.05$).

Results

Median hatching time, egg hatchability, and sac-fry mortality were not affected by exposure to Aroclor 1254 (Table 1). In addition, the relative rate of yolk absorption was not noticeably different between the control and treated fish. Mortality of the fry at 48 d was significantly greater than that in the control ($P < 0.05$) in only the highest concentration (13 μg/L). Mortality of fry continued to increase throughout the study and at 118 d was significantly higher for fish held at the three highest concentrations than for the controls (Table 1). Growth of the brook trout fry as indicated by weight was decreased significantly ($P < 0.05$) after 48 d of exposure to Aroclor 1254 concentrations of 1.5, 3.1, 6.2, and 13 μg/L (Tables 2 and 3). However, no significant differences in surviving fry weights were noted at the end of the 118-d exposure.

Vitamin C and hydroxyproline concentrations were significantly decreased in sac fry by Aroclor 1254 concentrations of 1.5, 3.1, 6.2, and 13 μg/L (Table 2). After 118 d of exposure, fry backbone development was impaired by Aroclor 1254 exposure (Table 3). Vertebral collagen concentrations were decreased significantly in fish exposed to 0.69, 3.1, and 6.2 μg/L; calcium concentrations were increased significantly in fish exposed to 0.69, 1.5, 3.1, and 6.2 μg/L; and phosphorus concentrations and hydroxyproline concentrations in the isolated collagen were decreased significantly by all Aroclor 1254 concentrations. The reason for collagen concentration not being affected by 1.5

TABLE 2. Effects of Aroclor 1254 on hydroxyproline and vitamin C concentrations in brook trout sac fry continuously exposed for 38 d and on weight of fry exposed for 48 d. Values represent the mean of 19–50 observations for weight and 8 pooled samples of 6 sac fry each for hydroxyproline and vitamin C determinations.

Aroclor 1254 concn (μg/L)	Fish wt (g)	Hydroxyproline (mg/g)	Vitamin C (μg/g)
0	0.065	1.71	139
0.43	0.062	1.59	144
0.69	0.068	1.50	150
1.5	0.056[a]	1.40[a]	105[a]
3.1	0.049[a]	1.16[a]	101[a]
6.2	0.049[a]	1.29[a]	100[a]
13	0.042[a]	1.29[a]	100[a]

[a]Significantly different from the controls ($P < 0.05$).

TABLE 3. Weight and backbone composition of brook trout fry continuously exposed to Aroclor 1254 for 118 d after hatching. Backbone components expressed as milligrams per gram of dried backbone, except hydroxyproline, which is expressed as milligrams per gram dried collagen. Values represent the mean of 6–20 observations for growth and 4–8 observations for bone analysis.

Aroclor 1254 concn (μg/L)	Fish wt (g)	Backbone composition (mg/g)			
		Collagen	Hydroxy-proline	Calcium	Phos-phorus
0	0.68	454	29	80	3.9
0.43	0.64	437	23[a]	77	2.4[a]
0.69	0.65	351[a]	21[a]	162[a]	2.5[a]
1.5	0.64	435	23[a]	159[a]	1.9[a]
3.1	0.52	386[a]	23[a]	215[a]	1.8[a]
6.2	0.74	397[a]	25[a]	297[a]	2.2[a]

[a]Significantly different from the controls ($P < 0.05$).

μg/L is not completely understood, but the decrease in hydroxyproline in this group indicates that collagen metabolism was altered.

One week after hatching, fry had accumulated Aroclor 1254 residues from 3700 times (1.6 μg/g) that in the lowest water concentration to 1100 times (14.0 μg/g) that in the highest water concentration (Table 4). At the time when significant fry mortality occurred among fry exposed to the highest concentration, or 48 d after hatching, whole body residues were 419 μg/g or 32 000 times the water concentration. At that time, fry with residues ≤182 μg/g were surviving. After 118 d of exposure, Aroclor 1254 had accumulated in the surviving fry from 40 000 to 48 000 times the water concentration and 50% mortality had occurred in fry having 284 μg/g whole body residues.

TABLE 4. Whole body residues (micrograms per gram) of Aroclor 1254 in brook trout fry during continuous exposure to Aroclor 1254. Values in parentheses are accumulation factors. ND = not determined.

Aroclor 1254 concn (μg/L)	Duration of fry exposure after hatch at			
	7 d	34 d	48 d	118 d
0	0.1	0.5	0.3	0.2
0.43	1.6 (3720)[a]	6.2 (14418)	8.9 (20697)	17 (39534)
0.69	1.8 (2608)	15 (21735)	ND	31 (44919)
1.5	3.2 (2133)	ND	ND	71 (47333)
3.1	7.5 (2419)	ND	ND	125 (40322)
6.2	13 (2096)	122 (19677)	182 (29354)	284 (45806)
13	14 (1076)	119 (9153)	419 (32230)	—[b]

[a]Accumulation factor = (fry residue ÷ water concentration) × 1000.

[b]100% mortality.

Discussion

Chronic toxicity studies with eggs and alevins of coho salmon (*Oncorhynchus kisutch*) have shown that water concentrations of 56 μg/L of Aroclor 1254 significantly reduced percentage hatch, and exposure to 15 μg/L for 6 wk reduced length and weight of coho salmon (Halter and Johnson 1974). Nebeker et al. (1974) reported that the no-effect concentration for mortality in fathead minnow (*Pimephales promelas*) fry and reproduction in adults was between 1.8 and 4.6 μg/L, which is similar to the no-effect concentration for mortality and growth of brook trout fry in the present study. Our results agree with those from other studies of chronic effects in that the earliest life stages of fish are the most susceptible to Aroclor 1254. Snarski and Puglisi (1976) reported that Aroclor 1254 at concentrations ≤0.94 μg/L did not affect growth, reproduction, or survival in adult brook trout exposed for 71 wk and did not influence growth or survival in offspring exposed for 90 d after hatch. Results from these studies concur with our findings that Aroclor 1254 concentrations ≤1 μg/L have no effect on growth of brook trout; however, our results show that bone development was affected.

The effects of Aroclor 1254 on growth, bone composition, and vitamin C metabolism in brook trout are similar to effects induced by the organochlorine insecticide toxaphene in brook trout (Mayer et al. 1975) and fathead minnows and channel catfish (*Ictalurus punctatus*) (Mayer et al. 1977a; Mehrle and Mayer 1975). Chronic exposure of these species to toxaphene resulted in reduced growth, decreased collagen concentrations, and increased calcium concentrations in the backbone. Mayer et al. (1978) reported that the increased use of vitamin C by the fish for liver detoxica-

tion of toxaphene appears to cause a functional deficiency of vitamin C in the backbone and reduced collagen synthesis in fingerling channel catfish. Since fish are unable to synthetize vitamin C (Chatterjee 1973; Wilson and Poe 1973), only finite quantities are available from yolk during the sac-fry stage and from the diet after yolk absorption, for detoxication, collagen synthesis, and other metabolic processes.

In the present study, the decrease in growth and in hydroxyproline and vitamin C concentrations in sac fry exposed to Aroclor 1254, and the later increased mortality in fry 10 d after yolk absorption, suggest that the PCB exposure induced competition between developmental and detoxication processes for the use of vitamin C similar to that reported for other chemicals by Mayer et al. (1977b). After 118 d of exposure, fish weights were not significantly different, but bone development was impaired by Aroclor 1254. The continued mortality probably negated the earlier differences in growth by eliminating the more susceptible fish, as was reported by Mayer et al. (1977a) in channel catfish exposed to toxaphene. Although the more obvious effects of Aroclor 1254, such as increased mortality and decreased growth, were less apparent in the older fish, the effects of Aroclor 1254 on bone development may cause the backbones to be more susceptible to fracture during periods of stress. This condition may adversely influence the ability of trout to adapt to various environmental challenges (Mayer et al. 1977b).

An application factor of 0.01 has been recommended in establishing water quality criteria for organochlorine insecticides (Committee on Water Quality Criteria 1973). This factor is derived by dividing the maximum acceptable toxicant concentration (MATC; the highest continuous toxicant concentration that has no adverse effect on growth, reproduction, and mortality) by the 96-h LC50 value (Mount and Stephen 1967). The 96-h LC50 of Aroclor 1254 for brook trout fry was $>100\ \mu g/L$. On the basis of an application factor of 0.01, the MATC should be in the range of $\geq 1\ \mu g/L$. The no-effect concentration of Aroclor 1254 for brook trout fry growth and mortality was 0.7 and $1.5\ \mu g/L$, respectively. However, phosphorus and hydroxyproline content in backbone collagen was decreased significantly in fish exposed to all concentrations, indicating that the application factor should be <0.01 for Aroclor 1254.

Residues of PCBs are ubiquitous in the aquatic environment, but their biological significance in fish is not known. Aroclor 1254 residues reported in various species of fish from across the United States ranged from 1.4 to $42\ \mu g/g$ (Walker 1976). Total PCB residues ranged from 3.5 to $47\ \mu g/g$ in lake trout (*Salvelinus namaycush*) from Lake Michigan (Willford et al. 1976) and from 0.10 to $5.1\ \mu g/g$ in various fish species in Canada (Graham 1976). In the present study, whole body residues of Aroclor 1254 in fry with impaired bone development ranged from $17\ \mu g/g$ in the lowest exposure to $284\ \mu g/g$ in fish exposed to $6.2\ \mu g/L$. The Aroclor 1254 residues in brook trout fry from the two lowest exposure concentrations (0.43 and $0.69\ \mu g/L$) were within the range of residues found in fish in the natural environment. This suggests that the effects on bone development are realistic indications of the adverse influence of Aroclor 1254 in brook trout, and perhaps in other species of fish, in the aquatic environment. However, studies of longer duration than the present one are needed if the significance of Aroclor 1254-induced alterations in growth and bone development and the residue dynamics of PCBs are to be fully understood.

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Permanent Sedimentation of Nitrogen, Phosphorus, and Organic Carbon in a High Arctic Lake[1]

L. DE MARCH

Department of Fisheries and the Environment, Fisheries and Marine Service, Freshwater Institute, Winnipeg, Man. R3T 2N6

DE MARCH, L. 1978. Permanent sedimentation of nitrogen, phosphorus, and organic carbon in a high arctic lake. J. Fish. Res. Board Can. 35: 1089–1094.

The sediments of Char Lake, Resolute, Northwest Territories, were studied to determine storage rates of nitrogen, phosphorus, and organic carbon which could be compared with retention estimates of N and P and primary production and respiration estimates for organic C. The storage rate for P ($0.018\ g \cdot m^{-2} \cdot yr^{-1}$) was 129% of measured retention, storage of N ($0.087\ g \cdot m^{-2} \cdot yr^{-1}$) was 43% of measured retention, and the $1\ g \cdot m^{-2} \cdot yr^{-1}$ storage estimate for organic C was 18% of the difference between primary production and respiration. Lacustrine sediments were found to be very unevenly distributed with accumulations from 7 cm to an estimated 3 m in the last 6200 yr and to vary in N, P, and organic C concentrations from place to place.

Key words: sedimentation, nutrient budgets, arctic, lake, carbon, nitrogen, phosphorus

DE MARCH, L. 1978. Permanent sedimentation of nitrogen, phosphorus, and organic carbon in a high arctic lake. J. Fish. Res. Board Can. 35: 1089–1094.

Nous avons étudié les sédiments du lac Char, à Resolute (T.N.-O.), pour en déterminer l'accumulation de N, de P et de C organique et la comparer aux estimations de la rétention de N et de P et à celles de la production primaire et de la respiration pour le C organique. L'accumulation de P ($0{,}018\ g \cdot m^{-2} \cdot a^{-1}$) est égale à 129% de la rétention mesurée; celle de N ($0{,}087\ g \cdot m^{-2} \cdot a^{-1}$) à 43%; et celle du C organique ($1\ g \cdot m^{-2} \cdot a^{-1}$) à 18% de la différence entre la valeur de la production primaire et celle de la respiration. La répartition des sédiments lacustres était très inégale, avec des accumulations variant entre 7 cm et 3 m au cours des 6 200 dernières années; la teneur en N, P et C organique y variait d'un endroit à l'autre.

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CHAR Lake, 74°42′N, 94°50′W, N.W.T., was the site of production and energy flow studies as part of the International Biological Programme from 1968 to 1973. The basic limnological features may be found in Rigler (1972). The physical and chemical limnology and the study area were described by Schindler et al. (1974), primary production by Kalff and Welch (1974), benthic production and respiration by Welch and Kalff (1974), and allochthonous inputs and losses by de March (1975).

This study provides estimates of permanent loss of N, P, and organic C (C_o) to the sediments of Char Lake.

The bottom of Char Lake can be divided into three major zones: rocky, moss, and silt (Welch and Kalff 1974) (Fig. 1). The rocky zone extends from the lakeshore to a depth of about 2.5 m, the maximum depth of ice scour, and covers an area of 15.5 ha or 29.4% of the lake area (Welch and Kalff 1974). In the shallow parts of this zone fine sediments are only between the cobble-sized rocks, but in the deeper parts fine sediments are thicker and can be cored.

The moss zone extends from the lower edge of the rocky zone to between 5 and 20 m deep. Generally the bottom is covered with a 10–20-cm-thick layer of moss, only the upper portions of which are living. There are mossless patches within the moss zone, especially on the west side of the lake. The total area covered by the moss zone is 12.6 ha or 23.9% of the lake area (Welch and Kalff 1974). Mossless patches in the moss zone cover 2 ha or 3.8% of the lake area (Rigler 1972).

The remaining 22.5 ha or 42.9% of the lake area constitutes the silt zone (Welch and Kalff 1974). Its surface is covered by algal mats, other growths of benthic algae, or is bare silty sediment.

Some areas (Fig. 1) have boulders with little fine sediment near them. They occur on steeper slopes of the lake bottom.

[1]Contribution No. 31 of the Char Lake Project.

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FIG. 1. Bathymetric map of Char Lake, N.W.T., showing the three bottom zones and the location of cores (numbered).

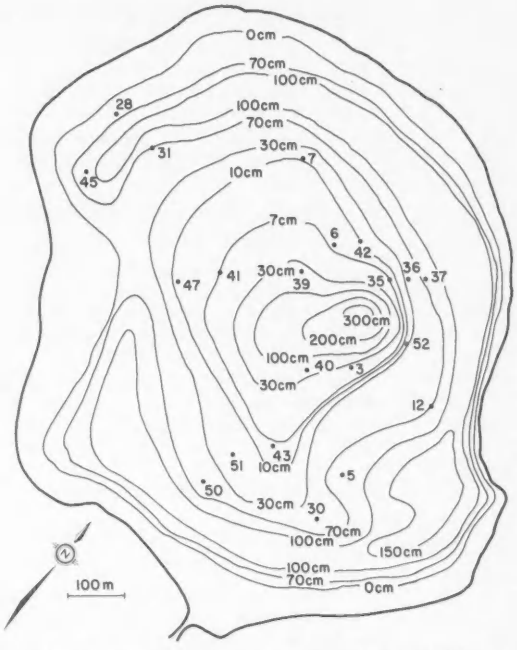

FIG. 2. Sediment thicknesses in Char Lake. Thicknesses are in centimetres and locations of cores used to construct the map are indicated.

Materials and Methods

Fifty sediment cores 25–104 cm long were obtained with a mini-Mackereth piston corer (Mackereth 1969) using acrylic core tubes of 5 cm inside diameter, and by SCUBA and manual driving, using the same tubes. Cores were obtained in June, July, and August of 1972 and 1973. Seven cores were from the lower rocky zone, 17 from the moss zone, and 26 from the silt zone.

In 32 cores sections were taken at 2-cm intervals to 10-cm and alternate 5-cm intervals to the bottom. In the remaining cores the intervals were 1 cm from 0–2 cm, 2 cm from 2–10 cm, and 5 cm to the bottom.

Each core section of known volume was weighed to determine its wet density. After homogenization and drying at 105°C, water contents were calculated as the loss of weight on drying. Sediment samples were then ground to a powder in a ceramic mortar and stored in glass vials.

For C, N, and P analyses, small samples (2–20 mg, depending on the analysis) were weighed out in preweighed aluminum pans on a Cahn Model G electrobalance. Samples were redried at 105°C and cooled in a desiccator before the final weight determination. All analyses were performed in duplicate and the mean value used.

Total C and N were measured on a Carlo Erba Model 1100 C-H-N analyzer, carbonate carbon (CO_3-C) was measured by the method of Stainton (1973), and C_o was obtained by difference. Total P was determined by the method of Stainton et al. (1974).

A small number of samples were subjected to X-ray diffraction analysis for the qualitative determination of the major mineral components in the sediments.

The recent sedimentation rate at the lake's deepest point (Z_m) was estimated by the ^{210}Pb dating method (G. Kipphut, Institute of Marine Sciences, University of North Carolina, Morehead City N.C. 28557, personal communication; see also Koide et al. 1973). Sediment thicknesses through the zones dated (2–8 cm) were recalculated at the density of the top 2 cm to give a sedimentation rate at that density. This value was recalculated to give a rate at the mean compacted density of the sediment (mean density from 8 to 83 cm).

Radiocarbon dates were not obtained; therefore, an age for the lake was determined from uplift data in the literature (Andrews 1968; Andrews et al. 1971; Blake 1970). Assuming that sedimentation rates have been constant and equal to recent rates since the formation of the lake, I calculated the sediment thickness at Z_m from the age of the lake and the ^{210}Pb sedimentation rate. Maximum sediment thicknesses in the moss zone were estimated from total core lengths and facies present.

To calculate the masses of C_o, N, and P in the sediments, a map of sediment thicknesses (Fig. 2) was drawn using the 22 cores that reached the lacustrine–marine interface. Areas between sediment thickness isopleths were measured by planimetry. Masses of each element were calculated using the mean concentration weighted by section thickness and the mean thickness and the area of sediment between isopleths. Doughnut-shaped areas were treated as rectangles with width equal to the mean radius of torus of equal area. The volume below the minimum thickness of an area (for

TABLE 1. Depths of the CL 2–3 and CL 3–4 boundaries in cores which reached the marine sediments. I = indistinct.

Core no.	CL 2–3 (cm)	CL 3–4 (cm)
3	13	24
5	44.5	80
6	6	8
7	I	0
12	I	69
28	I	65
30	42	57
31	62	70
35	I	7
36	20	35
37	45	84
38	10	25
39	21	40
40	15.6	34
41	I	8
42	I	8
43	I	8
45	51	57
47	I	10
50	29.5	40
51	18.2	24
52	6	10.2

example the sediment from 1 to 2 m deep in the area between 1- and 2-m-thick lines) was assumed to have a right triangular cross section, and the elemental masses were calculated accordingly. Concentrations of elements in sediments between the bottom of incomplete cores and the lacustrine–marine interface were assumed to equal concentrations in the lowest 30 cm of the cores.

Dividing the total masses of N, P, and C_o in the sediments by the estimated age of the lake gave storage rates averaged over the whole period of lacustrine sedimentation.

Results

With the exception of those obtained in moss beds all sediment cores had the surface layer intact. The moss was pulled aside in order to core the sediments below.

There were four facies identified in a number of cores and 22 cores penetrated the lowest layer (Table 1). The facies could be separated by color, texture, and physical and chemical characteristics (Fig. 3 shows a typical core).

In areas of no moss cover, cores exhibited a 1–2 cm orange-brown surface layer of high water content (CL 1). Below this, CL 2 was denser, grey, and in cores from deeper parts of the lake, laminated. CL 3, below a usually sharp transition, was finely laminated with bands of brown and black alternating with bands of white and grey. There were up to 10 band pairs·mm^{-1}.

CL 4 was a homogeneous, dense grey sediment which

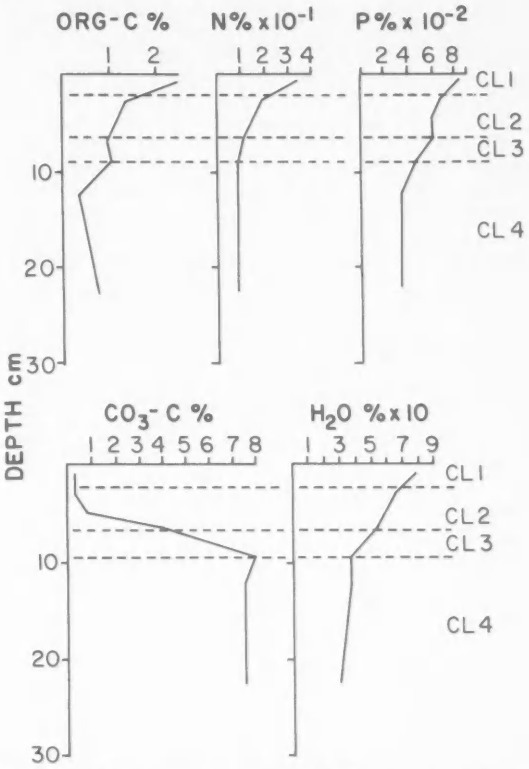

FIG. 3. Water content (percent of total weight), and concentrations of C_o, CO_3-C, N and P in core 6 from the silt zone (Fig. 2). Concentrations are percent of dry weight.

usually contained small stones and marine fossils such as spines of the sea urchin *Strongylocentrotus droebachiensis* and mollusc shells (e.g. *Astarte crenata*, *Cylichna scalpta*, and *Nuculana pernula*). Joined pairs of *N. pernula* valves were found in two cores, while other cores contained complete valves of other species with periostracum intact. The presence of marine fossils, some in living positions, indicates that CL 4 sediments are marine. CL 3 sediments fit the description of sediments laid down during the meromictic stage of Schleinsee (Geyh et al. 1974) and probably represent the time during which Char Lake was under tidal influence and the time it took to clear of seawater. CL 1 and 2 are sediments deposited since the clearing of seawater from Char Lake. CL 1 represents the oxidized sediments and CL 2 the reduced sediments.

In all cores sediments contained less water with increasing sediment depth. Concentrations of N, P, and C_o were generally low with higher levels in CL 1 and 3. The greatest changes in concentrations and water content occurred through the top 10 cm and through CL 3 (when CL 3 occurred below 10 cm) (Fig. 3).

TABLE 2. Average concentrations (percent of dry weight) of C_o, CO_3-C, N, and P in the three bottom zones of Char Lake and percent of total C_o stored in each zone.

Zone	C_o	$\%C_o$	CO_3-C	N	P
Silt	1.7	20.3	1.5	0.21	0.062
Moss	4.1	69.4	1.6	0.32	0.057
Rocky	5.1	10.3	2.3	0.32	0.057

TABLE 3. A comparison of permanent storage rates and mean concentrations of total sediment (TS), C_o, CO_3-C, N, and P calculated from a single deep-water core and from 50 cores. Rates are grams per square metre per year and concentrations are percent of dry weight. Rate error is rate for one core divided by rate for 50 cores.

	50 cores		Single core		Rate error
	Rate	Concn	Rate	Concn	
TS	30.7	100.0	255.0	100.0	8.3
C_o	1.0	3.2	3.0	1.41	3.0
CO_3-C	0.48	1.6	2.4	0.94	5.0
N	0.087	0.28	0.51	0.20	5.9
P	0.018	0.059	0.146	0.057	8.1

Phosphorus concentrations were fairly constant between lake bottom zones, but CO_3-C was highest in the lower rocky zone and N and C_o concentrations were highest in the moss and rocky zones (Table 2 — based on mean concentrations of all lacustrine sediments of all cores in each zone).

Carbonate-carbon concentrations were low in CL 1 and 2, but rose through CL 3 and were high in CL 4.

X-ray diffraction analysis of sediments in CL 1 and 2 confirmed the low concentrations of CO_3-C. There were small amounts of calcite and dolomite, but quartz was the major mineral. There were also traces of clay minerals. CL 3 and 4 contained predominately calcite and dolomite in approximately equal amounts. Quartz and traces of unidentified minerals were also present.

Two rock samples from the watershed proved to be calcite and dolomite, respectively (by X-ray diffraction). Both contained quartz. A soil sample from a stream delta contained mostly calcite with quartz and some dolomite.

A mean of maximum and minimum ages for the emergence level of Char Lake calculated from data in Andrews (1968), Andrews et al. (1971), and Blake (1970) was 6200 yr. This figure, based on currently available data, is $\sim \pm 3000$ yr (upper and lower estimates).

Figure 2 and the sedimentation data in Table 3 are based on the 6200-yr age and a sedimentation rate of 0.5 mm·yr^{-1} at 0.52 g·cm^{-3} at Z_m (^{210}Pb dating, G. Kipphut personal communication. Fig. 4).

Sedimentation results based solely on the core taken at Z_m, and not taking into account the physical and chemical differences between cores, are also given in Table 3 for comparison with the results based on 50 cores.

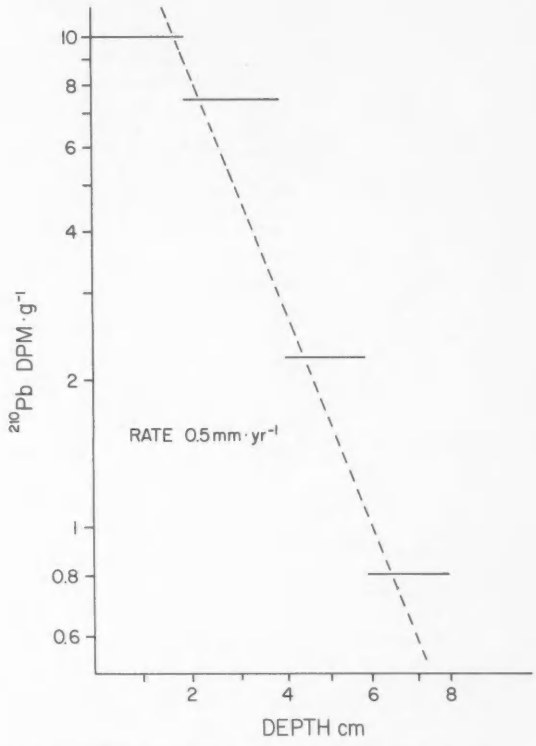

FIG. 4. ^{210}Pb data for core 2. The depth scale has been calculated to account for sediment density differences.

TABLE 4. Budgets for N, P, and organic C in Char Lake. All values are grams per square metre per year.

	C_o	N	P
Inputs[a]	2.5	0.326	0.025
Primary production[b,c]	21.1	—	—
Respiration[c]	15.9	—	—
Outflow[a]	2.1	0.122	0.011
Retention	5.6	0.204	0.014
Storage	1.0	0.087	0.018

[a]de March (1975).
[b]Kalff and Welch (1974).
[c]Welch and Kalff (1974).

Sedimentation for P agreed well with the difference between supply and outflow loss in the budget (Table 4) (129% of expected). Storage of N (Table 3) was 43% of the difference between supply and outflow loss (Table

4). Stored C_o (Table 3) was 18% of the C_o not accounted for by respiration and outflow loss (Table 4).

Discussion

Total sedimentation in Char Lake is similar to other lakes. The 0.5 mm·yr^{-1} sedimentation rate at Z_m is within the 0.45–0.6 mm·yr^{-1} range reported for the English Lakes District (Mackereth 1966), although the concentrations of organic C, N, and P are at the low end of the range reported by Brunskill et al. (1971). These are not unreasonable in a lake of low productivity in a watershed with low terrestrial production rates of 1–8 g organic matter m^{-2}·yr^{-1} (Arkay 1972).

Uneven sediment thicknesses were expected because in most lakes resuspension moves particles towards deeper water. Davis (1973) found that sediment thicknesses between different stations in Frains Lake varied by a factor of 10. Similarly in Char Lake, deep-water sediments have collected at a rate that is 8 times the mean accumulation rate (Table 3).

Because in only slightly shallower water ($\simeq$25 m) sediment thicknesses are considerably less (cores 3, 39, 40) than at Z_m this could indicate an error in ^{210}Pb dating or with some other factor. Further ^{210}Pb data could clarify this point.

The expected gradation from thin sediments nearshore to thickest sediments at Z_m as at Frains Lake (Davis 1973) was not found over most of Char Lake because of the presence of the moss zone. The moss acts as a sediment trap and the moss is buried in situ. The area just deeper than the moss zone is in a sediment shadow caused by the moss zone, and sediment accumulations there are minimal (Fig. 2). On the west side of the lake, where the moss zone is virtually nonexistent, sediments grade from thinnest near shore to thickest at Z_m as expected.

The differences in chemistry from place to place (Table 2) complicated the patterns of storage for individual elements. If this occurred in other lakes the direct measurement of storage would be difficult because of the large number of cores required for accurate estimates.

The drawback to the present study is that ^{14}C dates could not be obtained (due to a shortage of funds). Not having dated levels within cores precluded the determination of changes in sedimentation rates through the history of the lake. A comparison can only be made between long-term average storage rates and nutrient budget and production measurements made over 2 or 3 yr. The fact that the estimate of stored N was 43% of estimated retention does not negate the storage results. Nutrient inputs to lakes vary from year to year. For example P inputs to Char Lake were 0.016 g·m^{-2} in 1971 (Schindler et al. 1974), 0.027 g·m^{-2} in 1972 and 0.022 g·m^{-2} in 1973 (de March 1975) and retentions for these years were 0.012, 0.018, and 0.009 g·m^{-2}, respectively. The nitrogen storage estimate may also be low because of denitrification in the sediments.

In spite of all the possible errors the P storage estimate corresponds quite closely to retention estimates.

The estimate of C_o storage from production and respiration results (Table 3) was 6 times the direct estimate. Assuming the long-term average storage estimate is correct, there are three possible explanations for the discrepancy. First, the average storage of C_o was less than the present rate; second, production estimates for the lake are too high, and third, respiration estimates are too low.

Acknowledgments

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Seasonal Changes in Nitrogen Compartments of Lakes Under Different Loading Conditions

C. F.-H. Liao and D. R. S. Lean

Department of Fisheries and the Environment, Process Research Division, Canada Centre for Inland Waters, Burlington, Ont. L7R 4A6

Liao, C. F.-H., and D. R. S. Lean. 1978. Seasonal changes in nitrogen compartments of lakes under different loading conditions. J. Fish. Res. Board Can. 35: 1095–1101.

To develop a better conceptual picture for nitrogen dynamics in lake ecosystems, the seasonal changes in the concentrations of nitrate, ammonia, dissolved organic and particulate nitrogen were monitored in the water contained in three limnocorrals located in the Bay of Quinte, Ontario. Following the decline in summer particulate N, there was an increase in ammonia. This was followed by an increase in nitrate throughout the winter suggesting that nitrification was a significant process. To assess the potential contribution of the sediments, ammonia, nitrate, and dissolved organic N were measured for pore waters and total N, exchangeable ammonia, and nitrate in the solid fraction. The influence of nutrient addition was evaluated by comparing corral I, receiving no nutrient additions, with corral II to which phosphate was added (0.92 g P/m^2 per yr) and corral III which received the same amount of phosphate plus nitrate at an N:P weight ratio of 13. Increases and decreases in phosphorus, nitrogen, and chlorophyll *a* seemed to take place in all three corrals at the same time. There was a considerable delay in the response to nutrient enrichment, but the addition of nitrate and phosphate eventually resulted in a chlorophyll *a* level greater than that for phosphate enrichment alone. The importance of sediments as a net source of N for the planktonic community was insignificant, but the flux rate for nitrogen to and from the sediment was important. During the summer an input–output budget showed that nitrate loading plus N_2 fixation was approximately equal to the net change in the total combined N in the water. This implied that denitrification was not important, but over 12 mo most of the nitrogen that entered the system was lost; hence denitrification must be important at certain times. A lake ecosystem tends to compensate for low or high levels of NO_3 loading. Without the addition of nitrogen the requirement was partly met through nitrogen fixation and this can be enhanced by P enrichment.

Key words: ammonia, nitrate, nutrient kinetics, nitrogen fixation, denitrification, sediments

Liao, C. F.-H., and D. R. S. Lean. 1978. Seasonal changes in nitrogen compartments of lakes under different loading conditions. J. Fish. Res. Board Can. 35: 1095–1101.

Pour se faire une meilleure idée du cycle de l'azote dans les écosystèmes lacustres, les auteurs ont mesuré les variations saisonnières de la concentration des nitrates, de l'ammoniaque, des particules azotées et de l'azote organique dissous dans trois enceintes situées dans la baie de Quinte, en Ontario. Après une diminution du nombre de particules azotées en été, la concentration d'ammoniaque s'est élevée. Durant l'hiver, les nitrates ont augmenté, ce qui porte à croire qu'il se produit une importante nitrification. Pour déterminer si les sédiments prenaient part au cycle, on a dosé l'ammoniaque, les nitrates et l'azote organique dissous dans les eaux interstitielles, ainsi que l'azote total, l'ammoniaque et les nitrates mobiles dans la fraction solide. On a aussi évalué l'effet des éléments nutritifs en comparant les résultats de l'enceinte témoin I à ceux de l'addition de 0,92 g de P à phosphates par m^2 dans l'enceinte II, et à ceux de l'addition de la même quantité de phosphates que ci-dessus plus des nitrates en quantité 13 fois supérieure dans l'enceinte III. Les diminutions et les augmentations de phosphore, d'azote et de chlorophylle *a* semblent se produire au même moment dans les trois enceintes. L'effet des éléments nutritifs, qui s'est fait longtemps attendre, s'est traduit par une plus grande concentration de la chlorophylle *a* par suite de l'addition de phosphates et de nitrates par rapport à la seule addition de phosphates. Les sédiments, dont l'apport net de N au plancton a été dérisoire, ont cependant été le siège d'échanges importants d'azote. D'après le bilan estival, l'apport de nitrates plus la fixation de N_2 ont égalé approximativement la perte de N lié dans l'eau. La dénitrification n'a donc pas été considérable durant cette période.

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Toutefois, comme après 12 mo, la plus grande partie de l'azote ajouté avait disparu, il faut en conclure qu'une forte dénitrification se produit à d'autres moments. L'écosystème d'un lac cherche à s'opposer aux oscillations de l'apport de NO_3. Quand on n'avait pas ajouté d'azote, cet équilibre a été en partie atteint par la fixation de N_2, que l'addition de P est en mesure d'augmenter.

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THE amount and form of combined N which enters a lake ecosystem can control the algal biomass and alter the species composition. This latter point is particularly important from an aesthetic point of view: an equivalent density of phytoplankton distributed throughout the water column is less objectionable than a floating mat of Cyanophyta. In this investigation, we illustrate the seasonal transformations in the forms of N in a lake ecosystem, and the influence of the addition of nitrate (NO_3) along with phosphate (PO_4) on algal biomass. The importance of nitrogen fixation and sediment N as a source of N for the planktonic community was assessed. In addition, this paper provides the descriptive background for the measurements of N flux within the water column (Liao and Lean 1978).

The problems of sampling entire lakes (spatial heterogeneity, advection, uncertainty of amount and forms of N loading) were reduced by conducting experiments in enclosures (limnocorrals). This eliminates the problems of calculating N loading from a drainage basin. The sediments were not altered and their importance as a source or sink for N was assessed. Such systems can be manipulated by varying the nutrient loading conditions and comparisons made between the enclosures. The shapes were similar, hence morphometric differences minimized.

The experiments were conducted in the Bay of Quinte, a bay on the northern shore of Lake Ontario (Lean et al. 1975) through which flows much of the drainage of south-central Ontario. In the area where our experiments were conducted, the water was 4 m deep. It neither stratified nor became anoxic, but has been eutrophic for many years. No combined N was added to corrals I and II, but II did recieve phosphate enrichment. Since the sediments have a high (1.5%) total nitrogen content, it was thought that this site would offer an extreme example where sediment return could be assessed.

These experiments have particular relevance to those attempting input–output N-budgets for lakes. Without including estimates for the pathways of N-fixation and denitrification, serious errors may result. This paper, Liao and Lean (1978), and Lean et al. (1978) provide an integration of many of the important nitrogen pathways and are part of an overall program to relate nutrient fluxes, radiant energy input, and algal response.

Materials and Methods

The limnocorrals (Kepner Plastics, Torrence, California), shaped as equilateral triangles (area 25 m^2), consisted of a flotation collar which supported a skirt that extended from the surface to sediment (4 m) and continued 0.5 m into the sediments. The bottom was held down by a heavy chain embedded in the fabric and lateral movement was prevented by cables attached at the corners. Little exchange with the adjacent water occurred, even in storms where waves were up to 0.5 m. The total volume contained was approximately 100 000 L.

No nutrients were added to corral I. Corral II was enriched with P as H_3PO_4 (7.07 μg P/L per wk from July 12 to August 2, 1973; 14.14 μg P/L per wk thereafter until October 17, 1973); corral III was enriched with 7.07 μg P/L per wk plus nitrate in an N:P weight ratio of 13. During 1974 enrichment began on May 24 and continued to September 16 at the latter rate.

From July 1973 to September 1974, composite water samples (0.5, 1.5, 2.5, 3.5 m) were collected from the enclosures and adjacent water of the Bay of Quinte. Within 2 h 1 L was filtered through Whatman GF/C filters and the particulate and dissolved forms of nitrogen analyzed. The filtrate was acidified with 2 mL concentration H_2SO_4 and evaporated to ca. 100 mL. Half of the concentrated filtrate was subjected to modified semimicro Kjeldahl procedure (Bremner 1965) for total dissolved nitrogen. The other half was neutralized and analyzed for NH_4 and NO_3 by the steam distillation method (Bremner and Keeney 1965); NH_4 was determined by titration (McKenzie and Wallace 1954). The total dissolved organic nitrogen was calculated as the difference between the Kjeldahl and NH_4 analyses on the filtrate. Particulate N was determined by the semimicro Kjeldahl procedure (Bremner 1965). The techniques were chosen to be consistent with the subsequent ^{15}N-assimilation studies (Liao and Lean 1978).

Phosphorus analyses were conducted using the Dillon and Rigler (1974) modification of Strickland and Parsons (1972) technique. Prewashed Whatman GF/C filters were used instead of membrane filters since GF/C filters are used for particulate N and chlorophyll *a* analysis. Correlations with prewashed Millipore membrane filters were made and will be published later. Chlorophyll *a* was measured as described in Strickland and Parsons (1972).

Sediment cores (4.7 cm diam) were taken with a Kajak–Brinkhurst corer (Research Instruments Co., Guelph, Ontario) and subdivided into 2-cm sections. Pore water was collected by centrifuging the sediment at 10 000 rpm (12 000 *g*), for 10 min, then filtering through Whatman GF/C filters.

The sediment was air-dried, ground by mortar and pestle, and stored in an air-tight bottle for N analysis. Total N was analyzed by the semimicro Kjeldahl procedure. Sediment exchangeable NH_4 and NO_3 were extracted with 2 N KCl (1:50) and determined by the steam distillation method. Total sediment N was also determined routinely with a Carlo-Erba C–N analyzer. The cores were sectioned

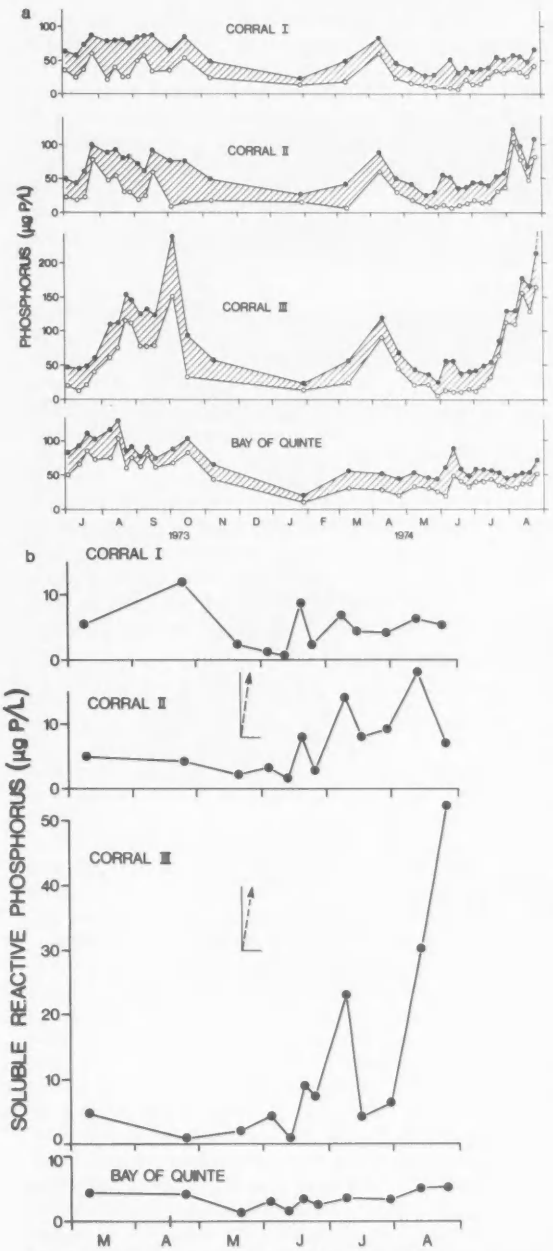

FIG. 1. a, Total phosphorus (-●-) unfiltered and filtered (-○-) using prewashed GF/C glass fiber filters. Shaded area represents particulate P; b, soluble reactive phosphorus for 1974; arrow (insert) represents start of enrichment and the slope represents rate of enrichment.

as above and centrifuged, but freeze-dried before being ground by mortar and pestle.

Results and Discussion

WATER COLUMN

The influence of nitrogen on lake trophic state is pronounced but cannot be fully appreciated without information on phosphorus and chlorophyll *a* concentrations. Although the phosphorus part of this investigation is presented elsewhere (Lean et al. 1975; Lean and Charlton 1976; Manning 1977; and other papers in preparation), some of the relevant information is given below.

An obvious omission in our experimental design was an experiment to evaluate the influence of nitrate or ammonia alone. These experiments are in progress and no prolonged enhancement in chlorophyll levels has been observed. It is the combined effect of nitrogen and phosphorus that causes substantial increases in biomass.

Although the same amount of P was added to corrals II (P only) and III (P + N), a higher total P concentration was reached in III (Fig. 1a). In fact, Corral II maintained a total P concentration closer to I (no P or N). What is even more surprising is that even though phosphate enrichment of II and III began in 1974, on May 24, the total P in the three corrals remained similar until near the end of July. Although differing in magnitude, the phosphorus changes in the three corrals appeared synchronized. All showed summer increases then declined to low (<25 µg P/L) in winter. Data to March 1975 (not presented here) also show these low winter values. Soluble reactive phosphate (SRP), measured only in 1974 (Fig. 1b), began to increase in III in June, declined in July, then increased at a rate equivalent to the rate of enrichment (see arrow Fig. 1b) and the total P increased even more rapidly. Mössbauer spectroscopy revealed that partial reduction of the sediment iron had occurred in III (Manning 1977).

The chlorophyll *a* (Fig. 2) was similar in all three corrals until August, then it increased just prior to, and proportional to, the phosphorus increase. It follows that phosphorus–chlorophyll correlations (Dillon and Rigler 1974) may be improved if the factors causing this delay were better understood.

The seasonal changes in the forms of N (Fig. 3) are similar in all three corrals and the Bay despite the fact that only III, and perhaps the Bay, received any NO_3 enrichment. Low NO_3 concentrations persisted throughout the summer, but from September 1973 the NO_3 concentration began to increase, reaching levels of 125–135 µg N/L in corrals I, II, and the Bay, and over 665 µg N/L in corral III by April 1974. From September 1973, the NH_4 levels increased rapidly (200–400 µg N/L), and then usually showed a slight decline throughout the winter. In I, this decline was equivalent to the nitrate increase. Nitrification is the obvious explanation for this observation. In April and May 1974, the nitrate levels declined again to typically low summer levels and did not correspond to an increase in particulate N. Instead, the particulate and the total N were also declining. This implies that denitrification may

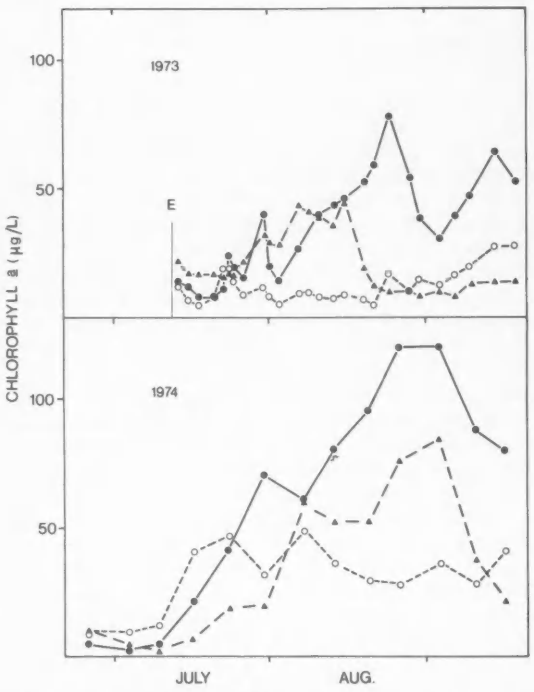

FIG. 2. Seasonal chlorophyll α analysis for corral I (-○-), corral II (-▲-), and corral III (-●-). E = enrichment.

be going on and illustrates the need for a bioassay to quantify this process. Alternatively, there may have been a net sedimentation of N.

Given typical monitor data, it would be quite impossible to know that corral III received the high NO_3 loading, while I and II received none. The high concentration (665 μg N/L) observed in March represents only 29% of that added in 1973. If the added NO_3 was not transformed and/or lost from the water column, the concentration of nitrate would have been 2300 μg N/L at this time. The amount added in 1974 would have further increased the NO_3 concentration to 5600 μg N/L.

During the summer the concentration of NH_4 remained constant, never much below 100 nor over 250 μg N/L. All corrals and the Bay were remarkably similar with corrals I and III increasing over II and the Bay during the winter. These data, like so many similar set of data derived from lake surveys (e.g. Claesson and Ryding 1975; Burns 1976), might be interpreted to mean that ammonia is less important as a source of N to the plankton than nitrate. These constant levels of NH_4, however, represent a quasi steady state where rates of regeneration and utilization are in balance. The demand for ammonia remains high and the uptake rates are rapid. Nitrate is comparatively unimportant unless ammonia becomes depleted (see Liao and Lean 1978).

The dissolved organic nitrogen (DON) is operationally defined as that fraction other than nitrite, nitrate, and ammonia that passes through a Whatman GF/C filter. However, it would not be surprising to find much of this fraction is of colloidal or small particle size. In the three corrals and the Bay, DON increased through July, August, and to mid-September 1973, and then followed a progressive decline to March 1974. A slight increase was found in corral II during March 1974. From April through mid-July 1974 the concentration started to increase rather irregularly from about 100 to 300 μg N/L.

Increases in DON parallel the increase in biomass, but the maximum biomass does not always correspond with the maximum DON. The ratio of DON to particulate N is surprisingly high, ranging from 1.2 in the summer to 4 in the winter. This represents an enormous pool of N that has yet to be chemically defined and we know little about its biological importance.

The striking feature of the seasonal pattern of changes in particulate N under different loading conditions is not the differences, but the similarities. The oscillations of the three corrals and (often) the Bay appear to be synchronized. Particulate N started to increase during April, declined late in May, then increased again in late summer. The initial increase in April was thought to be due partly to resuspension of sediments.

SEDIMENT

It was hoped that sediment analyses in winter and summer would reflect the influence of NO_3 enrichment and help explain any net seasonal changes in combined N in the water column. The amount and forms of N in the sediments were analyzed on August 21, 1974 and February 25, 1974. Two techniques were used to measure total particulate N, semimicro Kjeldahl and dry combustion at 900°C with a Carlo-Erba C–N analyzer. The results were similar, but the C:N values were obtained more frequently (duplicate cores twice a month in July and August). No seasonal trends or significant differences were found between the corrals. The percent N in the sediments varied little down to 10 cm and was in a range of 1.35–1.60 (mean 1.45% N).

Winter values for percent N are generally lower than those in summer (Table 1), but this cannot be considered a trend since the measurements were made too infrequently. No nitrate was ever found in the pore waters. This is not surprising since the sediments were anoxic and the Eh usually negative (Murphy and Lean 1975). In the top 6 cm pore water NH_4 increased from about 1 to 6 mg N/L compared to 0.1–0.3 in the water column. Interstitial ammonia continued to increase to about 9 at 18 cm, the maximum depth sampled. There was little difference between the corrals, but the winter levels were about 1 mg/L less than summer levels. Using the data for pore water volume per 4.7 cm diam core, this amount can be converted to concentration units in

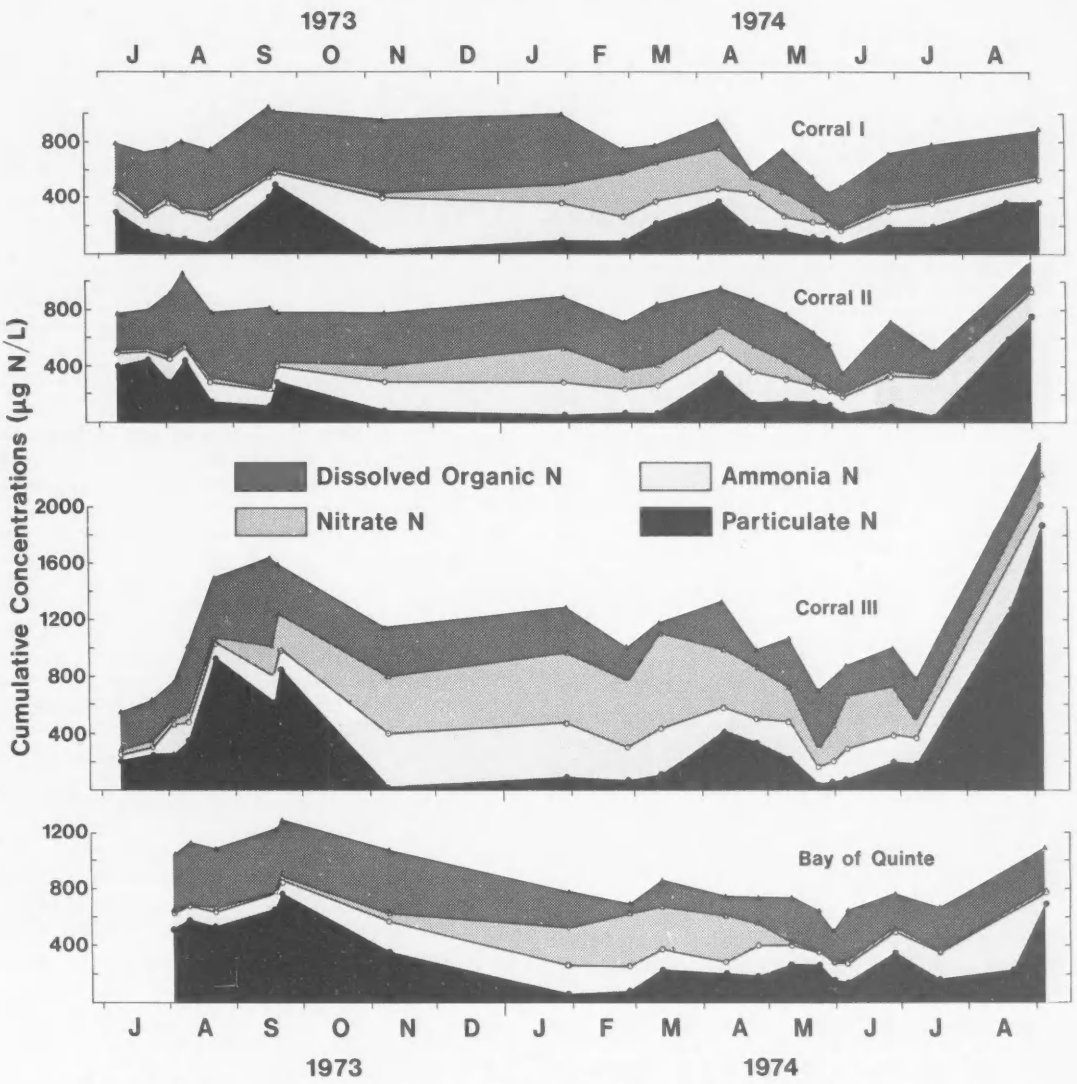

FIG. 3. Cumulative concentrations of particulate, ammonia, nitrate, and dissolved organic nitrogen.

the water column. The 1 mg/L decline in the 6 cm of sediments is an insignificant amount (1.08 g/corral or 10.8 μg N/L) in the water column. Forty to eighty percent of the combined N in the pore water was NH_4; the rest was lumped as DON. No pattern was obvious and no differences between corrals observed. In summary, there is an ammonia gradient in the sediments which suggests that it may be diffusing to the overlying water; however, it is the rate of formation of ammonia that is of critical importance. The quantity of sediment NH_4 is an insignificant source of N for the overlying water.

Estimates of exchangeable NO_3 and NH_4 were also made (Table 1). We thought that this may provide a useful index to estimate a pool of nitrogen potentially available for phytoplankton growth. Though NO_3 was never detected in pore water, some exchangeable-NO_3 (5–30 g N/g sediment) was present. However, nitrifying bacteria were present down to 18 cm (C. F.-H. Liao unpublished data) and conversion of NH_4 to NO_3 might have occurred during drying. There was more exchangeable-NH_4 than exchangeable-NO_3, but there was little difference between the corrals. There was an increase from the summer average of 81 to 134 μg N/g sediment in the winter. This (for the top 6 cm) amounts

TABLE 1. Sediment nitrogen analysis for corrals I–III in summer (S) and winter (W).

	Depth (cm)	I S	I W	II S	II W	III S	III W
% N	0–2	1.68–1.39		1.64–1.37		1.56–1.39	
	2–4	1.42–1.39		1.50–1.33		1.52–1.39	
	4–6	1.39–1.49		1.44–1.36		1.40–1.46	
Exchangeable NH_4-N (μg N/g)	0–2	93–127		84–115		78–119	
	2–4	69–132		91–115		82–143	
	4–6	57–153		93–136		80–175	
Exchangeable NO_3-N (μg N/g)	0–2	22–14		22–7		9–16	
	2–4	18–18		16–11		11–20	
	4–6	14–14		24–9		5–14	
Pore water NH_4-N (mg N/L)	0–2	1.4–0.8		1.7–1.4		3.2–1.8	
	2–4	4.3–2.3		3.0–2.1		5.2–3.8	
	4–6	3.2–3.1		5.6–3.5		6.3–4.4	
Pore water DON (mg N/L)	0–2	1.2–1.4		0.9–0		1.8–2.4	
	2–4	3.6–0.9		1.6–1.7		1.0–1.8	
	4–6	4.3–2.7		0.9–1.6		1.1–2.3	
		Core wt (g)		Pore water vol (mL)			
Avg measurements for 4.7-cm core	0–2	2.26		25.4			
	2–4	2.93		23.2			
	4–6	3.53		23.0			

to only 6.7 g/corral or 67 μg NH_4/L in the overlying water. However, a value of 100 μg N/g represents only 0.01% of the sediment weight and this is only 0.7% of the sediment nitrogen. The pore water DON and NH_4 together may reach as high as 8 mg N/L in our sediments (88.5 μg/g or 0.6% of the sediment N). Obviously, the identification of the forms of N in the sediments and rates of conversion to nitrogen potentially available for algal growth and rates of diffusion of the overlying waters must be a worthwhile objective for future research.

NITROGEN FLUX

In this study, little, if any, net flux of N occurred from the sediments. The changes in NH_4 and exchangeable-NH_4 can represent a combined input of only about 100 μg N/L to the water. Indeed, this may account for part of the ammonia increase that occurred in September.

Byrnes et al. (1972), however, claimed that the surficial layer of sediment (0–4 cm) would serve as an immediate source of NH_4 to the overlying waters, and the nitrogen in as much as 16 cm of sediment can ultimately be considered available. Since their experiments were done by adding excess NH_4 to the sediment, the results are difficult to extrapolate to natural conditions. In lakes, complex organic material sinks to the sediment and its subsequent decomposition cannot be quantified from experiments such as those described by Byrnes.

Despite the fact that there may be no net influx from the sediments, there is, however, movement to and from the sediments. These rates were estimated using sediment traps and the data corrected for sediment resuspension (M. N. Charlton 1975, CCIW, Burlington, Ont., personal communication; Lean et al. 1978). These samples were collected weekly and the summer monthly averages are given below:

	Dry wt (g/m^2 per d)		Nitrogen (g/m^2 per d)		% N	
	July	Aug.	July	Aug.	July	Aug.
I	1.81	2.83	.034	.054	1.90	1.90
II	2.23	2.69	.046	.052	2.04	1.92
III	2.24	2.79	.051	.089	2.28	3.20

The concentration of total combined N in the water columns can be integrated and expressed as grams per square metre. This amount divided by the sedimentation rate provides an average retention time for N. The reciprocal ($\times$ 100) is the percent sedimenting per day. The July–August retention time was 52–94 days and sedimentation rates 1.1–1.9%/d. The surficial sediments contained ca. 1.5% N; whereas the sedimenting material was 1.9–3.2% N. The particulate material in the water column contained up to 9% N. It follows that either N is released during sedimentation, or the fraction of the particulate material that settles out has a low percent N. Furthermore, additional losses of N occur in the sediments. This process is commonly termed decomposition, but the specific conversion requires investigation and definition.

The N inputs to the corrals include the 0.105 g N/m^2 per d of NO_3 loading to corral III. Precipitation inputs were negligible. N_2-fixation (Lean et al. 1978) was 12, 60, and 6 g/corral per summer for corrals I, II, and III, respectively. The processes that contribute to the inputs and outputs (g/m^2 per 2 mo) are summarized below:

		I	II	III
Inputs:	Loading	0	0	6.5
	N_2-fixation	0.5	2.4	0.2
	Sediment return	?	?	?
Outputs:	Sedimentation	2.72	3.04	4.44
	Denitrification	?	?	?
Net change		0.8	2.4	6.4

The net change data from July 1 to August 31 (from Fig. 3) were +200, +600, and +1400 (μg N/L) for corrals I, II, and III, respectively. These were converted to areal units (g/m^2).

For July and August it would appear that the loading and N_2-fixation were approximately equal to the net change. This implies that sedimentation and sediment return were in balance and that denitrification was a relatively unimportant process. However, an amount of NO_3 was added to corral III over 2 yr equivalent to 5600 μg N/L. Over the year, denitrification must have

been important, but where and when remains unknown.

The phosphorus loading to corral II was 14.14 μg P/L per wk or 0.5 g/m^2 per 2 mo. The N_2-fixation estimate provides an input of N_2 which gives an N:P ratio of 5, surprisingly close to that required for algal growth. On the other hand, no phosphorus was added to corral I and the N_2-fixation reduced to only about 20% that for corral II. This illustrates the potential for lake ecosystems to obtain nitrogen through N_2-fixation when required.

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Nitrogen Transformations Within the Trophogenic Zone of Lakes

C. F.-H. Liao and D. R. S. Lean

Department of Fisheries and the Environment, Process Research Division, Canada Centre for Inland Waters, Burlington, Ont. L7R 4A6

Liao, C. F.-H., and D. R. S. Lean. 1978. Nitrogen transformations within the trophogenic zone of lakes. J. Fish. Res. Board Can. 35: 1102–1108.

The uptake rates for ammonia and nitrate were measured in limnocorrals and in the adjacent water in the Bay of Quinte, Ontario. Even though the net change of nitrate exceeds that for ammonia over the summer, it is ammonia that is the most significant source of N for the plankton. The uptake rates were light dependent; in the dark uptake of nitrate was reduced 60–95% and ammonia by 40–70%. The uptake of N is not correlated to net production but to gross production. These rates were integrated with sedimentation, N release by zooplankton excretion, and N_2-fixation. Although N_2-fixation provides only a small amount of the daily N requirement compared with ammonia uptake, ammonia is rapidly recycled and the N_2-fixation is the vital process controlling the amount of this essential nutrient in systems where it is in short supply (corral II). Zooplankton grazing and sedimentation (decomposition) can account for much of the ammonia regeneration, but direct release of ammonia from the plankton appeared to be important.

Key words: ammonia, nitrate, ^{15}N kinetics, zooplankton grazing, nitrogen fixation

Liao, C. F.-H., and D. R. S. Lean. 1978. Nitrogen transformations within the trophogenic zone of lakes. J. Fish. Res. Board Can. 35: 1102–1108.

Nous avons mesuré la vitesse d'absorption de l'ammoniaque et des nitrates dans des enceintes lacustres et dans les eaux adjacentes de la baie de Quinte, en Ontario. Même si le changement net de la concentration des nitrates pendant l'été était supérieur à celui de l'ammoniaque, cette dernière est tout de même la source la plus importante de N pour le plancton. Les vitesses d'absorption dépendaient de l'éclairement; dans l'obscurité, elles étaient réduites de 60 à 95% pour les nitrates, et de 40 à 70% pour l'ammoniaque. L'absorption de N n'est pas reliée à la production nette mais plutôt à la production brute. Les vitesses mesurées ont été intégrées à la sédimentation, l'excrétion de N par le zooplancton et la fixation de N_2. Même si la fixation de N_2 ne compte que pour une faible partie des besoins journaliers en N, comparativement à l'absorption d'ammoniaque, cette dernière est recyclée rapidement et la fixation de N_2 constitue le processus vital par lequel les quantités de cet élément nutritif essentiel sont maintenues dans les milieux où les approvisionnements sont insuffisants (enceinte II). Le broutage par le zooplancton et sa sédimentation (décomposition) sont les sources les plus importantes de régénération de l'ammoniaque mais l'excrétion directe par le plancton semble aussi jouer un rôle important.

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The relative importance of the nutrients essential to the growth and composition of algae in lake waters remains poorly understood. The availability of nitrogen profoundly influences and, in some cases, controls algal biomass; however, information such as the rates of inorganic nitrogen turnover, the relative nutritional importance of the various nitrogen forms, and the effect of concentration on the rates of assimilation are scarce. With the recent introduction of ^{15}N tracer methodology for measuring the nitrogen transfer rates, some information has been obtained (MacIsaac and Dugdale 1969; Dugdale and Dugdale 1965; Brezonik 1968), but many confusing artifacts still exist.

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Measurements of some of the key N-transformations were made within the trophogenic zones and related to inputs and outputs as measured by Liao and Lean (1978). In this study the uptake of nitrate and ammonia by the plankton in lakes was measured by the ^{15}N technique and integrated with estimates for primary production, N_2-fixation, sedimentation (decomposition), zooplankton excretion, and net change in particulate N. Methodological problems that exist for estimating these processes are discussed.

Materials and Methods

The descriptive limnology which serves as a background for this investigation has been presented earlier (Lean et al. 1975; Lean et al. 1978; Liao and Lean 1978).

Uptake rates of ammonium and nitrate nitrogen were carried out in situ using the ^{15}N-isotope technique similar to that described by MacIsaac and Dugdale (1969). Samples were collected by mixing equal subsamples from 0.5, 1.5, 2.5, and 3.5 m. A known quantity of ^{15}N-labeled $(NH_4)_2SO_4$ (95.6%) or KNO_3 (96.6%) was introduced into 1-L glass bottles containing 900 mL of water and then returned for incubation at 1 m. Uptake measurements were also made in light bottles at 1 and 3 m and in the dark. Generally, the added substrate concentration was 200 μg N/900 mL and the incubation time was 24 h; nitrate and ammonia uptake rates were measured at added substrate concentrations of 30, 50, 100, and 200 μg N/L, and incubated for shorter periods.

After incubation, the water was filtered through GF/C glass filters (Whatman) to collect the particulate nitrogen fraction and dried for later analysis. Particulate nitrogen was determined by semimicro Kjeldahl procedure (Bremner 1965) using the acid titration method (McKenzie and Wallace 1954). The samples were then redistilled in dilute acid and stored in a tight vial for isotopic analysis according to the procedure outlined by Bremner (1965). The instrument used was a Micro-Mass spectrometer (Vg-602c).

Measurements of primary production were made with the bottle O_2 method (Vollenweider 1968). Zooplankton grazing was estimated with the technique described by Haney (1971).

Results and Discussion

Additions of ^{15}N-NO_3 and ^{15}N-NH_4 drastically increase the in situ concentration, particularly those systems with a N deficiency. This could alter the steady state to such a degree that N values have little meaning. Furthermore, if insufficient isotope is added, the substrate will decrease during the incubation. This dilemma is further complicated by the time, labor, and cost of processing a sufficient number of samples, at a series of substrate levels and at several light levels, to provide data relevant to the actual process.

Additional problems stem from using enclosed samples. Whenever parts of a community are isolated, the "open system" becomes a "closed system" and soon begins to change. An assumption is made that the supply of nutrients other than nitrogen inside the bottle is similar to that outside the bottle. Particularly with long incubations, this may be a serious problem.

TABLE 1. Influence of substrate concentration on the uptake of inorganic N. Samples were incubated in 1-L bottles for 8 h (1100–1900), on July 31, 1974. Numbers in parentheses denote rates calculated with the in situ nitrate or ammonia plus that added.

	Substrate added (μg N/L)	In situ concn (μg N/L)		Uptake rate (μg N/L per h)	
		NH_4-N	NO_3-N	NH_4-N	NO_3-N
I	30	150	5	2.76 (16.6)	0.29 (0.39)
	50			—	0.43 (0.52)
	100			5.54 (13.8)	—
	200			7.34 (12.9)	0.30 (0.31)
II	30	150	10	1.68 (10.1)	0.25 (0.33)
	50			2.43 (9.72)	0.37 (0.44)
	100			3.75 (9.38)	0.56 (0.62)
	200			4.83 (8.45)	0.47 (0.50)
III	30	185	10	3.61 (25.8)	3.43 (10.3)
	50			5.26 (24.7)	4.75 (10.5)
	100			7.57 (21.6)	5.16 (8.26)
	200			17.27 (33.3)	5.43 (7.07)
Bay	30	110	60	2.71 (12.6)	0.82 (0.96)
	50			4.45 (14.2)	0.87 (0.96)
	100			5.21 (11.0)	0.84 (0.88)
	200			6.27 (9.72)	0.69 (0.71)

Since the phytoplankton biomass is one of the major factors in determining the utilization rate of nitrogen, it would seem reasonable to express the uptake rate as per unit biomass. Dugdale and Goering (1967) expressed their values as a fraction of total particulate N and called it a growth rate (coefficient). This is analogous to activity coefficients used in ^{14}C-primary production measurements. Since a significant portion of the particulate carbon and nitrogen is not living, this can be misleading. Nevertheless, in the absence of any improved estimate of biomass N, we also expressed the amount incorporated as a fraction of the particulate N.

Influence of Substrate Concentration

The uptake rates of ammonium-N and nitrate-N at different added substrate levels are shown on Table 1.

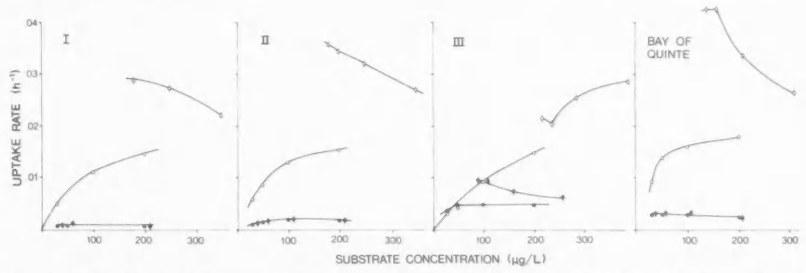

FIG. 1. Uptake rate for ammonia (○) and nitrate (●) as a function of added substrate levels. The measurements corrected for the natural substrate level are shown with a vertical line. The corrections for nitrate only differ for corral III, but those for ammonia differ in all experiments. Incubation time was 1100–1900, July 31, 1974. To compare the three corrals and the Bay the uptake rate was divided by particulate N.

TABLE 2. The effect of light intensity on the uptake of inorganic nitrogen (September 17, 1973).

	Depth (m)	Intensity (% I_0)	Uptake rate[a] (μg N/L per d)		Particulate N[b] (μg N/L)	
			NH_4-N	NO_3-N	(1)	(2)
I	1	10	269	129	670	558
	3	0.2	126	33	450	440
	1	0	105	23	512	462
II	1	32	125	27	167	160
	3	3	53	5	166	117
	1	0	37	2	168	108
III	1	10	93	58	740	983
	3	0.2	35	7	664	531
	1	0	28	1	673	—
Bay	1	20	252	60	933	714
	3	2	185	29	840	801
	1	0	150	18	833	634

[a]Corrected for in situ concentration. For corrals I, II, III, and the Bay, the levels were 86, 113, 155, and 93 μg N/L for ammonia, and 4, 0, 205, and 2 μg-N/L for nitrate. Samples were incubated for 24 h (1200–1200).

[b](1) and (2) are particulate-N for ammonia and nitrate incubation, respectively.

When the in situ concentration was neglected and the rates plotted against added substrate concentration, smooth curves were obtained, similar to that expected for Michaelis–Menton (M/M) kinetics (Fig. 1). When the in situ ammonia concentration was included (Fig. 1), the curves often showed a decline in rate with increasing substrate levels. The natural substrate level was so low for nitrate in corrals I, II, and the Bay that little change was observed when it was included, but corral III showed the inverse slope. It seems unlikely that less N would be taken up at the higher concentrations unless the plankton became saturated with N. A more likely explanation is that used by Rigler (1966) to explain similar kinetics for phosphate uptake. He assumed that the analytical values for phosphate were too high and by substituting lower values the curves take the shape expected for M/M kinetics. Perhaps the methods for ammonia analysis should also be carefully reexamined.

In addition to the problems of substrate analysis, note that if the substrate level is reduced during the incubation the calculated value would be too high. Future work should include analysis before and after incubation. Previous works (Prochazkova et al. 1970) reported increases and decreases in in situ ammonia during the incubation.

Nitrate uptake by plankton in corral III (Fig. 1) is greater than in the other corrals. This may be a characteristic of the predominant algae present (*Microcystis aeruginosa*) or indicate that nitrate reductase has been activated from the regular inputs of nitrate. The uptake of ammonia in corrals I and II exceeds that for nitrate by more than tenfold. However, in corral III ammonia uptake was occasionally only 2–3 times higher. If no corrections are made for in situ ammonia, it is surprising to find that the asymptote (V_{max}) for the ammonia curve is similar for all corrals and the Bay (i.e. $\simeq 0.2 \cdot h^{-1}$).

We should be cautious in extending M/M kinetics from enzyme–substrate studies to single organism–nutrient relationships and then to lake-community nutrient studies.

INFLUENCE OF LIGHT INTENSITY

Composite water samples were incubated with ^{15}N-ammonia or nitrate at 1 and 3 m in the dark (Table 2). Rates of ammonia uptake at 1 m were reduced by 53, 58, 62, and 27%, for corrals I, II, III, and the Bay, respectively, when incubated at 3 m. Rates were further reduced an additional 5–10% when the samples were incubated in the dark.

Light had an even greater influence on nitrate uptake. The 1-m rates were reduced by 74, 83, 87, and 52%, in corrals I, II, III, and the Bay, respectively, when incubated at 3 m. The rates were even further reduced in

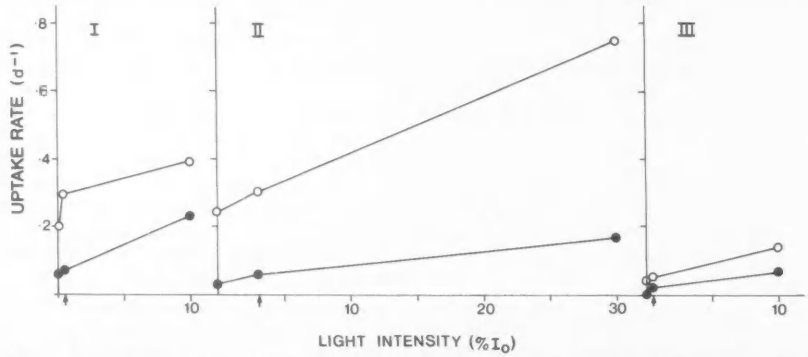

FIG. 2. Ammonia (○) and nitrate (●) uptake rates as a function of light intensity. Arrows (↑) denote 3-m incubation.

TABLE 3. Seasonal changes in ammonia and nitrate uptake.

	In situ concn (μg N/L)		Uptake rate (μg N/L per d)		Uptake rate constant (d^{-1})	
	NH_4	NO_3	NH_4	NO_3	NH_4	NO_3
Sept. 17, 1973						
I	86	4	268	129	0.40	0.230
II	113	0	125	26.7	0.36	0.170
III	155	205	93	58.0	0.13	0.059
Bay	93	2	252	60.0	0.27	0.084
June 5, 1974						
I	102	8	16	2.5	0.24	0.037
II	133	7	20	4.6	0.31	0.058
III	126	238	25	6.4	0.32	0.089
Bay	133	5	99	24.0	0.52	0.150
July 17, 1974						
I	158	16	128	14.7	0.25	0.031
II	281	3	112	9.3	0.76	0.088
III	179	102	217	45.6	0.40	0.098
Bay	188	0	190	31.4	0.46	0.087
Sept. 4, 1974						
I	167	21	115	10.4	0.28	0.026
II	182	16	96	1.5	0.12	0.002
III	178	178	139	1.5	0.06	0.001
Bay	88	7	159	23.8	0.20	0.032

[a]In situ concentration before ^{15}N-substrate was added.
[b]Uptake rates measured after adding 200 μg ^{15}N-NH_4 or ^{15}N-NO_3 to 900 mL lake water and incubating 24 h.

TABLE 4. Comparison of primary production and N-uptake (at 1 m).

	Production (μg C/L per d)		N-uptake (μg N/L per d)		Ratio C:N
	Gross	Net	NH_4	NO_3	
June 7, 1974[a]					
I	135	−62	16	2.5	7.1
II	187	−180	20	4.6	7.6
III	234	−174	25	6.4	7.5
Bay	375	−179	99	24.0	3.0
July 25, 1974[b]					
I	979	+720	128	14.7	6.9
II	433	−28	112	9.3	3.6
III	657	+327	217	45.6	2.5
Bay	598	−35	190	31.4	2.7
Sept. 4, 1974					
I	1264	+1062	115	10.4	10.0
II	1464	+976	96	1.5	15.0
III	1116	+384	139	1.5	8.0
Bay	1452	+427	159	23.8	8.0

[a]N-uptake measured on June 5.
[b]N-uptake measured on July 17.

the dark. In corral III uptake was almost completely suppressed.

From the light uptake relationships (Table 2) the rate per unit particulate N for the three corrals can be compared under similar light conditions (Fig. 2). The slopes of the curves are similar and although much more work is required, this may provide a useful index for variation due to light intensity. Syrett and Fowden (1952) were the first to postulate that the uptake of nitrate and ammonia is related to the photosynthetic activity of the plankton. Alternatively, the apparent reduced assimilation rates in the dark may result from the release of ammonium (Prochazkova et al. 1970).

For routine analysis, the choice of 1 m seemed to be reasonable, since the average depth of the euphotic zone during the 1973 summer was 3.5, 3.5, and 2.8 m for corrals I, II, and III, respectively. Nevertheless, the light level in our experiment at 1 m was 10, 32, and 10% of that at the surface for corrals I, II, and III, respectively. Future work must include a more complete range of incubations as a function of light for NH_4 and NO_3 uptake measurements.

Seasonal Changes in Nitrate and Ammonia Uptake Rates

Uptake rates were measured on four occasions other than July 31 (Table 3). The amount of nitrogen taken up per unit particulate N permits comparisons between corrals that minimize biomass differences. To reduce the complications of correcting for diurnal light changes, the samples were incubated for 24 h.

In all the experiments, the uptake rate of NH_4 was greater than for NO_3, especially in corrals II and III on September 4, 1974. Ammonium can be immediately incorporated into amino acids, whereas nitrate must first be converted to ammonia by a reaction requiring energy and the enzyme nitrate reductase. If the nitrate concentration is low, a delay prior to uptake may result, since a period is required for the induction of the enzyme (Healey 1973). This indicates that further experiments should include sampling throughout the 24 h.

Reexamination of the seasonal analysis of the forms of N in the corrals (see Liao and Lean 1978, fig. 3) indicated that as summer progresses, nitrate is depleted and ammonia remains relatively constant. This seems to be a typical pattern for lake surveys (Burns 1976; Schindler et al. 1973). The data from this study suggest that an amount of ammonia is taken up equivalent to 5–10 times that for nitrate. Prochazkova et al. (1970) claim that if the ammonia concentration exceeds 160 μg N/L, little nitrate will be used. This is consistent with the results from only about half of our experiments.

We attempted to relate the data for nitrate and ammonia uptake (Table 4) to primary productivity. (Often the measurements were not made on the same day, but this will not alter the general interpretations.) The uptake of N was taken as the sum of that for nitrate and ammonia. This too can be criticized but no pro-

TABLE 5. Particulate N and zooplankton grazing coefficients.

	I	II	III
June 5, 1974			
Particulate N (total) (μg N/L)	123	63	92
(<30 μm) (μg N/L)	47.4	42	46
Grazing coefficient (d^{-1})	0.41	0.33	0.48
July 17, 1974			
Particulate N (total)	520	217	988
(<30 μm)	198	121	108
Grazing coefficient	0.21	0.18	0.44
Sept. 4, 1974			
Particulate N (total)	398	980	2633
(<30 μm)	223	466	343
Grazing coefficient	0.24	0.14	0.13

cedure exists to calculate the "true" N uptake in lakes. Usually the nitrate contribution is small. Primary production was measured with the oxygen method. Bottles were incubated at 1 m for 2–3 h. The net production (i.e. growth) on June 7 was negative. In other words, respiration exceeded photosynthesis. The uptake of N can only be positive and hence cannot be related to net production. It does, however, compare quite well with gross production (i.e. total photosynthesis not corrected for respiration losses) and a very reasonable C:N ratio of 7–7.5 was obtained for the three corrals. The low C:N ratio of 3 for the Bay suggests that about half the N uptake is related to the production process.

No productivity data were obtained on July 17; therefore the N uptake results of July 17 were compared with productivity measured on July 25. Again, reasonable C:N ratios exist only when compared with gross photosynthesis. Slight changes in incubation depth can cause significant errors. For example, if 0.5-m productivity data were used instead of those for 1 m, the C:N ratios of 3.6, 2.5, and 2.7 are increased to 6.0, 4.7, and 5.1.

The September 4 data represent the beginning of a period of decline but a high biomass still existed. The C:N uptake ratios were 8.0–15. The measured C:N ratios of the particulate material was 7.5 in July, but gradually declined to 5 by September (unpublished data). The values obtained for the ratio of uptake of C to N vary between 2.5 and 15 with an average of 6.2. These data are insufficient to establish clearly a close coupling of N uptake and gross production, but are encouraging enough that more experiments along similar lines should be conducted. However, these observations are puzzling.

The day-to-day particulate N changes very little compared with the influx of N. We know too that total community respiration and photosynthesis are almost in balance. Since the measured uptake of N correlates with gross production there must be losses in nitrogen proportional to respiration of carbon. (Even though the ^{15}N estimates may be high due to enhancement by enrichment, this does not invalidate this argument.)

Attempts were made to account for the N losses from the plankton other than direct release and to assess the relative importance of zooplankton grazing and sedimentation decomposition (Table 6).

Many assumptions are made which are still unresolved in the measurement of zooplankton grazing and sedimentation. This, plus the uncertainty of the techniques used to measure the processes for nitrate and ammonia uptake and N_2-fixation, makes this analysis tenuous. Nevertheless, it is a beginning. Haney's (1971) zooplankton grazing technique involves feeding the zooplankton a ^{32}P-labeled yeast (*Rhodotorula*). This is done in situ in a plexiglass 10-L cylinder. After 10 min the radioactivity incorporated in the zooplankton is measured and the volume filtered to obtain this activity calculated. This allows one to calculate a grazing rate coefficient for the fraction of the water column filtered per unit time. In our experiments an amount of water equivalent to 15–48% was filtered free of particles < 30 μm diam each day (Table 5). After removing the larger particles by 30-μm Nitex screens, particulate

TABLE 6. N-Uptake and release estimates (μg N/L per d).

	June 5, 1974			July 17, 1974			Sept. 4, 1974		
	I	II	III	I	II	III	I	II	III
Uptake									
NH_4	16	20	25	128	112	217	115	96	139
NO_3	2.5	4.6	6.4	14.7	9	45.6	10.4	1.5	1.5
N_2-fixation	trace	3.5	—	2.0	3.0	0.5	4.0	4.0	trace
Total	18.5	28.1	31.4	145	124	263	129	102	141
Release									
Zooplankton grazing	9.7	6.9	11.0	20.7	11.0	23.6	26.8	32.6	22.3
Sedimentation	2.6	3.5	5.0	5.1	15.8	8.7	35.7	15.7	137.7
Total	12.3	10.4	16	25.8	26.8	32.3	62.5	48.3	160
Net change in particulate N	<1.0	<1.0	<1.0	+21.4	+12.3	+82.6	+3.3	−21	−158
Ammonia release (est.)	+6.2	+17.7	+15.4	98	85	148	63	75	139

material $< 30\ \mu m$ was collected and analyzed on the C–N equipment. It is obvious that this fraction is much less than the total particulate N and if some animals do eat the larger material, the importance of zooplankton grazing is underestimated by 1.5–7 times. No corrections were made for diurnal changes. It has been established that increases occur near sunrise and sunset (Haney and Hall 1975). For the Bay of Quinte, this increases the rate by about double at these times (J. F. Haney, University of New Hampshire, Durham, N.H., personal communication). A final assumption was that the assimilation efficiency was 50%. This represents a minimum estimate and it could be as high as almost 100%. This would cause the estimate for zooplankton regeneration to be doubled. The regeneration rate via zooplankton grazing is equal to the particulate N concentration ($< 30\ \mu m$) times the zooplankton grazing coefficient times the assimilation efficiency (assumed to be 50%).

Sedimentation rates were measured by Charlton (1975, unpublished data). He developed a technique to correct for sediment resuspension. Lean et al. (1975) provided ratiotracer data that were in good agreement with Charlton's estimate for phosphorus sedimentation. Consequently, we think this estimate is reliable. Recalling that Liao and Lean (1978) showed that the losses to the sediments are balanced by sediment return, we have assumed that particulate N settles out and ammonia is returned. This is effectively a significant regeneration mechanism. Measurements were made weekly but the rates used here were the average of the 2 wk prior to the experiment.

Fixation of N_2 was estimated by the acetylene reduction technique (Lean et al. 1978). Measurements were made from May to October, every 2 wk, except during July and August when the bioassays were run at least once per week. Incubation times were ½ to 4 h depending on the fixation rate and corrections made for changes due to diurnal and light intensity changes. The periphyton initially seemed to fix more than the plankton, but during peak periods the plankton were responsible for most of the N input by this process.

Nitrogen is, of course, taken up and released, but the net change in particulate N was significant during certain periods. This was estimated by taking the slope of the particulate N vs. time curve. Values given by Liao and Lean (1978, fig. 3) for particulate N were measured too infrequently for this analysis. Consequently particulate N measured with the dry combustion C–N analysis was used (unpublished data). These were collected weekly during the entire study except during the winter when the samples were collected only every 1–2 mo.

The N uptake and release processes are summarized in Table 6. We recognize that the ammonia and nitrate uptakes could be higher than the true N uptake due to the stable isotope enrichment. The in situ uptake is less than V_{max} and our enrichment was 200 μg N/L. We suspect too that the long incubations could have caused induction due to nitrate enrichment and, furthermore, that the rates could have been reduced, not only because of the closed-bottle techniques, but also the long incubations used. This, however, minimized the corrections required for changes in light intensity each day.

As in any mass balance calculation, the input equals output plus accumulation. In each case, our inputs (uptakes) are greater than the output (release) plus accumulation (net change in particulate N). This may be due to too high an estimate for NH_4 uptake, or too low an estimate for zooplankton grazing, or (the explanation we favor) the direct release of NH_4 from the plankton. This estimate is given at the bottom of Table 6 and is a process that deserves more attention in future experiments. Following the example of Prochazkova et al. (1970), bioassays for ^{15}N uptake should include estimates for change in ammonia concentration.

The uptake of ammonia is the principal influx of N. The nitrate influx is only 10–20% of that for ammonia and N_2-fixation is even less. This gives the false impression that N_2-fixation is unimportant, but when we realize the speed that ammonia is recycled, then this influx, which is also a net input to the system (Liao and Lean 1978) is in fact the most important source of N for corrals I and II (receiving no NO_3 enrichment). Surprisingly the uptake of NO_3 in corral III for September 4 (1.5) is less than the loading rate (26.3 μg N/L per d).

Zooplankton grazing causes a release of 10–50% of the N taken up, but this rate could easily be doubled if some of the assumptions prove to be incorrect.

Sedimentation (decomposition) is extremely variable and accounts for little regeneration in the July example (4–14% of the uptake), but more in June (14–17%) and September (30–110%). There was little net change in biomass in June; July was a period of rapid growth; in corrals II and III biomass declined in September. In all examples there was a net release of N (presumably ammonia) which could not be accounted for. This could also be due to errors in any or all of the process estimates.

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Rates of Mortality, Growth, and Biomass Production of *Lymnaea palustris* During Chronic Exposure to Lead

U. BORGMANN, O. KRAMAR, AND C. LOVERIDGE

Department of Fisheries and the Environment, Fisheries and Marine Service, Canada Centre for Inland Waters, Great Lakes Biolimnology Laboratory, Burlington, Ont. L7R 4A6

BORGMANN, U., O. KRAMAR, AND C. LOVERIDGE. 1978. Rates of mortality, growth, and biomass production of *Lymnaea palustris* during chronic exposure to lead. J. Fish. Res. Board Can. 35: 1109–1115.

Lymnaea palustris, a freshwater snail, was exposed to lead in a continuous-flow system from newly laid eggs until reproductive maturity. Lead, at concentrations as low as 19 $\mu g \cdot L^{-1}$, caused a significant increase in the mortality rate, although the growth rate was not affected. The lead-induced mortality was proportional to the lead concentration raised to the exponent 2.5. The analysis of mortality as a rate function was found superior to the use of the more conventional LC50 (concentration resulting in 50% mortality). A plot of the logarithm of the lead-induced mortality against log lead concentration is linear and has the same slope as plots of double logarithms of the inverse survival at any one time versus log lead concentration or log time versus log LC50 or log LT50 (time to 50% mortality) versus log lead concentration. The mortality rate, unlike the percent mortality, is independent of time and can be combined directly with the growth rate to estimate the biomass production rate. A 50% drop in the rate of biomass production was observed at 36 $\mu g \cdot L^{-1}$ and a drop to 0 production at 48 $\mu g \cdot L^{-1}$. Lead uptake in whole snails was found to be proportional to the lead concentration in the water, giving a concentration factor of 8500 on a dry weight basis.

Key words: snails, lead, toxicity, biomass production, bioaccumulation

BORGMANN, U., O. KRAMAR, AND C. LOVERIDGE. 1978. Rates of mortality, growth, and biomass production of *Lymnaea palustris* during chronic exposure to lead. J. Fish. Res. Board Can. 35: 1109–1115.

Nous avons exposé une hélice d'eau douce, *Lymnaea palustris*, au plomb dans un système à flot continu, depuis l'oeuf nouvellement déposé jusqu'aux individus ayant atteint la maturité sexuelle. A des concentrations aussi faibles que 19 $\mu g \cdot L^{-1}$, le plomb cause une augmentation significative des taux de mortalité, bien que la croissance ne soit pas affectée. La mortalité déclenchée par le plomb est proportionnelle à la concentration de plomb élevée à la puissance 2,5. Nous constatons que l'analyse de la mortalité comme fonction taux est supérieure à la méthode conventionnelle de la CL50 (concentration causant une mortalité de 50%). Si l'on porte le logarithme des mortalités déclenchées par le plomb contre le log de la concentration de plomb, on obtient une droite dont la pente est la même que les graphiques de doubles logarithmes de l'inverse de la survie en tout temps versus le log de la CL50 ou le log du TL50 (temps requis pour une mortalité de 50%) versus le log de la concentration de plomb. Contrairement au pourcentage de mortalité, le taux de mortalité est indépendant du temps et peut être combiné directement avec le taux de croissance pour estimer le taux de production de biomasse. Nous observons une baisse de 50% dans le taux de production de biomasse à 36 $\mu g \cdot L^{-1}$ et une baisse à 0–48 $\mu g \cdot L^{-1}$. Nous constatons que l'assimilation du plomb par des limnées entières est proportionnelle à la concentration de plomb dans l'eau, ce qui donne un facteur de concentration de 8 500 par rapport au poids sec.

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THERE is considerable concern about lead as a possible environmental pollutant. The Water Quality Board of the International Joint Commission between Canada and the United States has attempted to set objectives for lead, and other metals, in the Great Lakes. Below the set levels it is believed that these substances will not be harmful to people or aquatic life. Lead also appears on the list of substances of concern under the recent Environmental Contaminants Act in Canada. However, information on lead toxicity, which could be useful in setting water quality standards is limited. Although some studies on long-term effects on fish have been done (e.g. Davies et al. 1976), very little information on the effect of chronic exposure to lead is available for invertebrates. This study was conducted to determine

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the effect of long-term exposure to low levels of lead on growth, survival, and biomass production in the freshwater snail *Lymnaea palustris*.

Materials and Methods

All experiments were conducted using Burlington city tap water, which originates in Lake Ontario. Chlorine was removed by passage through a charcoal filter and the temperature was adjusted to 21 ± 2°C. Over the test period the water had a mean pH of 7.8 (ranging from 7.2 to 8.4), alkalinity (as $CaCO_3$) of 88 mg·L^{-1} (83–92), hardness (as $CaCO_3$) of 139 mg·L^{-1} (135–144), and conductivity of 356 μmhos·cm^{-1} (345–370). Lead was added as $Pb(NO_3)_2$ using a proportional dilutor modified from Mount and Brungs (1967). Water samples for lead analysis were taken approximately biweekly, resulting in measured lead concentrations (mean ± SD) of 3.8 ± 1.7 (control), 12 ± 3, 19 ± 2, 31 ± 6, 54 ± 13, and 97 ± 30 μg·L^{-1}. Five replicate test chambers were used at each concentration, consisting of square plexiglass compartments holding 1 L of water each. The flow rate per chamber was about 20 mL·min^{-1}. Loss of animals through the overflow was prevented by nylon mesh screening.

Snails were collected from Crawford Lake, a small lake about 20 km north of Burlington, Ont., in September 1976 and again in June 1977. They were kept in aquaria at room temperature and were fed commercial fish food flakes and lettuce. Egg masses were obtained by removing all previous eggs present in the tanks and collecting those laid within the next 24 h. After the eggs in each mass were counted, they were randomly placed in one of the test chambers, one egg mass per chamber. The number of eggs exposed per concentration ranged from 186 to 279 in the first experiment and from 114 to 136 in the second. In the first experiment, using eggs collected from adults obtained in September, the number of snails per test chamber was reduced to 10 (50 per concentration) 1 wk after hatching, and length and live weight were measured for each snail as soon as they were large enough to handle. None of the young were removed in the second experiment, which was shorter in duration and designed to measure survival only, using eggs collected from adults obtained the following year.

Lead analysis was performed on all snails from each concentration that survived to 120 d after hatching. The shell and the soft tissues were separated and dried at 60°C, followed by ashing at 500°C and digestion in nitric acid. Lead in water was determined as total lead from unfiltered samples. (The water was relatively free of particulates due to filtration by the city of Burlington and by passage through a charcoal filter in the laboratory before use.) Lead concentrations in the tissue and water samples were determined by atomic absorption spectroscopy by the Water Quality Branch at the Canada Centre for Inland Waters according to the procedures outlined in the *Analytical Methods Manual*, Inland Waters Directorate, Water Quality Branch, Ottawa (1974).

Results

Mean egg hatching time (for eggs that hatched), about 13 d, was unaffected by lead concentrations. Hatching success was >95% in all concentrations up to and including 31 μg·L^{-1}, in the first and second experiment. At 54 μg·L^{-1}, 79% hatched in the first and 62% in the second experiment. Although the eggs were still alive about 1 mo after being laid, only one egg in the first and none in the second experiment hatched at 97 μg·L^{-1}. The cumulative survival up to 120 d after hatching is shown in Fig. 1. Between 100 and 120 d after hatching, a large number of egg masses were observed at those concentrations still containing live snails. Hence the exposure period covered a full life cycle and reproduction depended on survival from egg to adult and not on the egg laying ability of exposed snails.

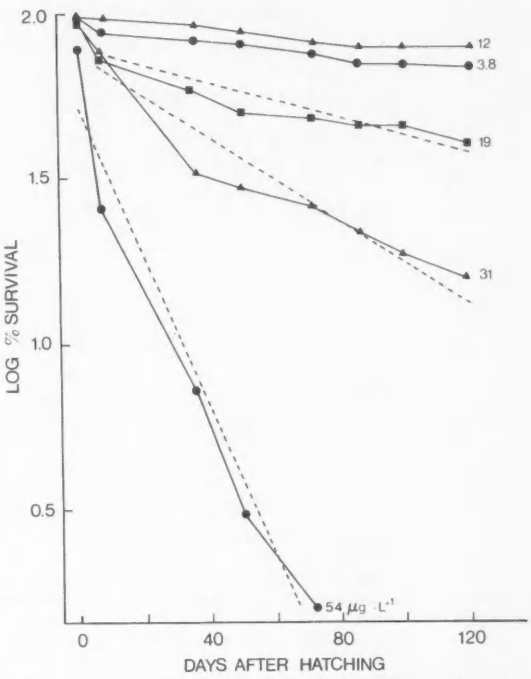

FIG. 1. Cumulative percent survival of snails as a function of days after hatching at various lead concentrations. Dashed lines are obtained from regression analysis. Numbers indicate lead concentration in water.

The mortality rate (m) at each concentration was calculated with the equations

$$(1)\quad dN/dt = -mN,$$

$$(2)\quad \ln N/N_0 = -mt,$$

where N is the number of snails alive, N_0 is the initial number of snails, and t is the time in days. The rate m is equal to the slope of the dashed lines in Fig. 1, multiplied by 2.303 (to convert to natural logarithms), and was obtained by regression analysis of log survival against time. These rates, calculated over a 120-d period, represent average mortality up to the age of reproductive maturity. Small variations in the rate, such as a slightly higher mortality rate of young (up to 50 d

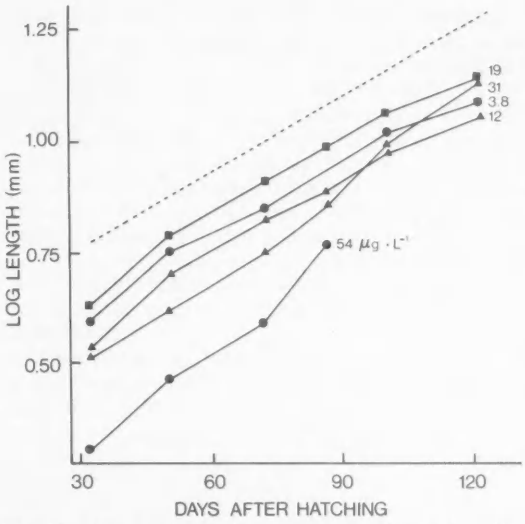

FIG. 2. Average length of snails as a function of days after hatching at various lead concentrations. A line of slope indicating a growth rate for length of $0.0132 \cdot d^{-1}$ is included for comparison. Numbers indicate lead concentration in water.

after hatching) at 19 and 31 $\mu g \cdot L^{-1}$, appear to occur.

Since length could be measured more accurately in smaller snails than weight, growth was determined in terms of length and is shown in Fig. 2. The growth rate is unaffected by lead up to 19 $\mu g \cdot L^{-1}$. Although the initial size is smaller, the growth rate appears to be slightly greater at 31 and 54 $\mu g \cdot L^{-1}$, since the data in Fig. 2 are average sizes for all surviving snails and not growth rates for individual animals. If small animals are more sensitive to lead, as suggested above for 19 and 31 $\mu g \cdot L^{-1}$, then selective mortality would result in a more rapid increase in average size of exposed snails than expected on the basis of growth of individual animals. Hence the growth rate of individual animals is believed to be independent of the lead concentration and was calculated from the lengths of snails at 3.8 and 12 $\mu g \cdot L^{-1}$ (where low mortality occurred). The growth rate for length (k) was calculated with

$$(3) \quad \ln L/L_0 = kt,$$

where L is the length, L_0 the initial length, and t the time in days. The rate k, indicated by the dashed line in Fig. 2, was obtained from regression analysis of log length versus time and was equal to $0.0132 \cdot d^{-1}$ (1.32% increase in length per day).

To obtain growth as a function of weight, we performed a regression analysis of log weight against log length on control snails ($n = 39$). This gave a slope (± 1 SE) of 2.87 ± 0.03. The growth rate for weight (g) was calculated from

$$(4) \quad \ln W = 2.87 (\ln L),$$

$$(5) \quad \ln W/W_0 = gt,$$

where W is weight. Combining equations 3, 4, and 5 gives $g = 2.87k = 0.0379 \cdot d^{-1}$ (3.79% increase in weight per day).

The data on survival and growth can be combined to give the total biomass production. Biomass is equal to the number of snails alive times the weight per snail and hence,

$$(6) \quad B = NW,$$

$$(7) \quad \ln B/B_0 = rt = (g - m)t,$$

where B is the biomass and r is the rate of biomass production.

The mortality rate m ($\pm 95\%$ confidence intervals from regression analyses), growth rate g, and production rate r are shown in Fig. 3, plotted as log rate against log lead concentration. The rate m at 12 $\mu g \cdot L^{-1}$ is not significantly different from m for 3.8 $\mu g \cdot L^{-1}$, and hence control mortality (m') was estimated from the average of these two values ($m' = 0.00244 \cdot d^{-1}$). The mortality due to lead was determined by assuming that the total mortality rate (m) was equal to the control mortality rate (m') plus the lead-induced mortality rate (m''). Subtracting control mortality from total mortality gave the estimates of lead-induced mortality shown as open circles in Fig. 3. Since these values fall on a straight line it appears that lead-induced mortality is proportional to lead concentration raised to some exponent (n) and hence,

$$(8) \quad m'' = C[Pb]^n,$$

where [Pb] is the lead concentration in micrograms per litre and C and n are constants. The complete equation for mortality rate as calculated from the data in Fig. 3 is given by

$$(9) \quad m = m' + m'' = 0.00244 + 2.28 \times 10^{-6}[\text{Pb}]^{2.5}.$$

The curve labeled m in Fig. 3 is given by equation 9 and is equal to the sum of the horizontal line m' and the diagonal line m''. The only point that does not fit neatly on this theoretical curve is at 12 $\mu g \cdot L^{-1}$. Equation 9 is consistent in form with general equations for biological rates as determined from theoretical considerations of multienzyme systems (Borgmann 1974).

The effect of lead on biomass production is given by combining equations 7 and 8.

$$(10) \quad r = g - m = 0.0355 - 2.28 \times 10^{-6}[\text{Pb}]^{2.5}$$

and is also shown in Fig. 3. The maximum production rate is $g - m' = 0.0355 \cdot d^{-1}$. Several important concentrations are indicated in Fig. 3. At a lead concentration of 48 $\mu g \cdot L^{-1}$, m is equal to g and therefore $r = 0$ and the production rate is nil. At higher concentrations, biomass production is negative, since mortality is greater than growth. At a lead concentration of 36 $\mu g \cdot L^{-1}$, m'' is equal to r and hence r is equal to one half the maximum production rate. Finally the lowest concentration

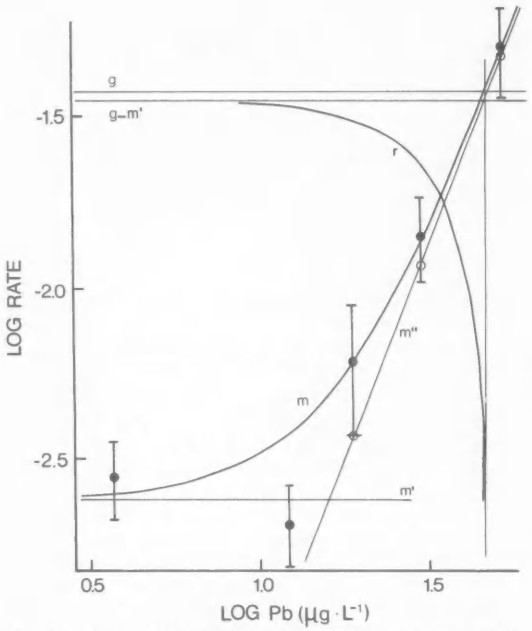

FIG. 3. Growth (g), total mortality (m), control mortality (m'), lead-induced mortality (m''), and biomass production (r) rates as a function of lead concentration. Vertical bars indicate 95% confidence limits.

tested showing significant lead-induced mortality was 19 $\mu g \cdot L^{-1}$, and the highest concentration tested showing no significant lead-induced mortality was 12 $\mu g \cdot L^{-1}$. Therefore, the total concentration range from no effect to complete elimination of production (12–48 $\mu g \cdot L^{-1}$) is very narrow. The narrowness of this range is due to the high value of n in equation 8. The significance of the exponent n can be demonstrated by comparing the lead concentration resulting in half maximum production ($r = m'' = \frac{1}{2}(g - m')$) with the concentration resulting in no production. The ratio of the mortality rates at these concentrations is

$$(11) \qquad \frac{m'' \text{ at } r = 0}{m'' \text{ at } r = m''} = \frac{[\text{Pb at } r = 0]^n}{[\text{Pb at } r = m'']^n} = 2,$$

and hence

$$(12) \qquad \frac{[\text{Pb at } r = 0]}{[\text{Pb at } r = m'']} = 2^{1/n} = 2^{1/2.5} = 1.32.$$

The concentration giving no production is thus only 1.32 times greater than the concentration resulting in a 50% drop in production rate. If the exponent n were equal to 1, positive production would still be possible at lead concentrations up to twice as high as that causing a 50% drop in production rate.

The concentrations of lead in the flesh (soft tissues) and the shells of the snails are shown in Fig. 4, along

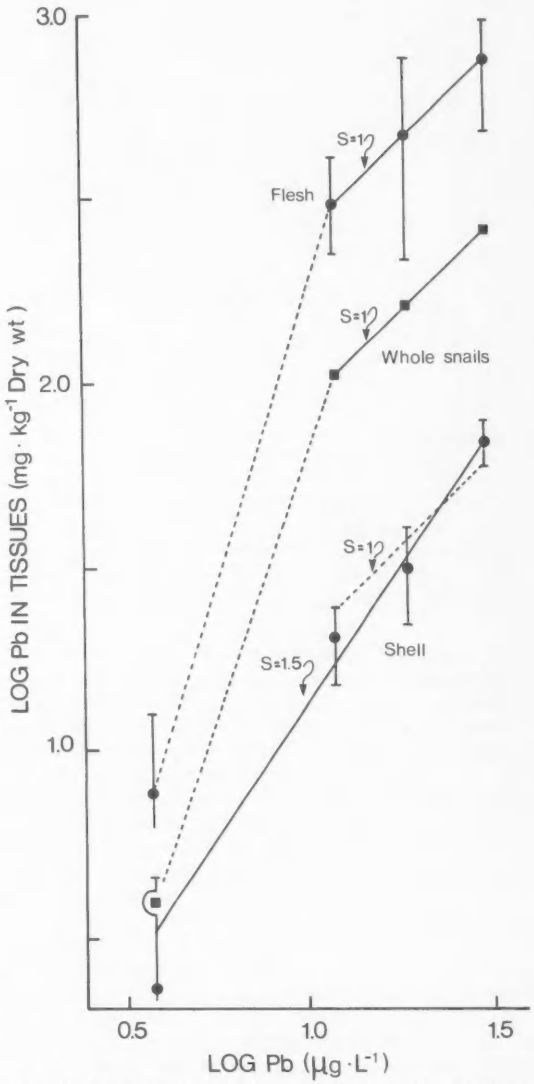

FIG. 4. Lead concentration in snail flesh, shells, and whole animals as a function of lead concentration. Vertical bars indicate 95% confidence limits. The slope of the lines is indicated by s.

with 95% confidence intervals at each concentration. Lead levels in whole snails, also shown in Fig. 4, were calculated using the flesh and shell levels and a flesh dry weight equal to 29% of the total dry weight of the snails (the ratio of flesh to shell dry weight per snail was not observed to vary with lead concentration). Although most of the dry weight of the snail is shell, the majority of the total lead content was found in the flesh (e.g. 81% at 31 $\mu g \cdot L^{-1}$). The relationship between

whole body lead levels and lead in the water is therefore similar to the relationship between flesh lead levels and lead in water. A single line of slope 1.5 can be drawn through all the points for the shell lead levels, but this is not possible for the flesh. Lead levels in the flesh and in whole snails appear to be proportional to the concentration in the water for all concentrations except the control. This might indicate that the lead occurring in the water before our additions is in a form that is less readily accumulated by snails than the lead added. Since lead accumulation is approximately proportional to lead in the water, and not to lead concentrations raised to the exponent 2.5, toxicity is not proportional to lead uptake.

The relationships between tissue lead levels and lead exposure as shown by the solid lines in Fig. 4 can be expressed by the following equations,

$$(13)\quad Pb_f = 24[Pb],$$

$$(14)\quad Pb_s = 0.40[Pb]^{1.5},$$

$$(15)\quad Pb_w = 8.5[Pb],$$

where Pb_f is the level in the flesh, Pb_s in the shell, and Pb_w in the whole snail (each expressed as milligrams Pb per kilogram dry weight of tissue), and [Pb] is the lead concentration in the water (micrograms per litre). Hence the concentration factor for flesh is 24 000, for whole snails is 8500, and for shells is dependent on lead concentration in the water. (This is equivalent to concentration factors of 3100 for flesh and 2500 for whole animals on a wet weight basis). Since these snails were exposed to lead for their entire development, it is assumed these tissue levels are in equilibrium with the water.

The toxicity of lead to the snails may be expressed in terms of tissue levels by combining any of equations 13, 14, or 15 with equation 10. For example, biomass production rate as a function of whole body lead levels is given by

$$(16)\quad r = 0.0355 - 2.28 \times 10^{-6}(Pb_w/8.5)^{2.5} = 0.0355 - 1.08 \times 10^{-8}(Pb_w)^{2.5}.$$

Although equations 13 and 15 only hold for the range of lead concentrations in water above 12 $\mu g \cdot L^{-1}$ (Fig. 4), equation 16 can be used over the entire concentration range, since at concentrations below 12 $\mu g \cdot L^{-1}$ the value of m'' becomes very small and r is approximately equal to 0.0355 and is independent of lead exposure. Theoretically, equation 16 could be used to estimate the reduction in the rate of biomass production in *L. palustris* due to lead exposure in the field, at least at temperatures near 20°C.

Discussion

The above investigation of the effect of lead on *L. palustris* does not use probit analysis and the estimation of an LC50 (concentration killing 50% of the animals in a given time period). Instead, mortality is expressed as a rate m, which unlike the LC50, is independent of time and is in units comparable with the growth rate. Toxicity can therefore be expressed by its effect on the biomass production rate. This is a more meaningful analysis of toxicity, since the lead concentration resulting in a mortality rate high enough to affect production is dependent on the growth rate of the organism (i.e. $r = g - m$). Thus a high toxicant-induced mortality in one species may be harmless if the growth rate is sufficiently rapid, whereas the same mortality rate in a slow-growing species might completely eliminate production. Hence the toxicity of lead to the snail *L. palustris* is demonstrated by a 50% reduction in production rate at 36 $\mu g \cdot L^{-1}$ and a drop to 0 production at 48 $\mu g \cdot L^{-1}$. A useful reference point in this snail might be the lead concentration resulting in a 20% drop in production rate (from 0.0355 to 0.0284 $\cdot d^{-1}$), which occurs at 25 $\mu g \cdot L^{-1}$ of lead ($m'' = 0.0071$), since over a 120-d period (approximate age to reproductive maturity) this would result in a final biomass in exposed snails of 43%, or a little less than half that of control snails.

Two basic assumptions are made in the above calculations. The first is that the rate of change of the number of snails N, weight W, and biomass B are proportional to N, W, and B respectively (i.e. equations 1 and 2, 5 and 7). If this assumption is valid, then the data in Fig. 1 and 2 should give straight lines as plotted. This assumption seems to hold reasonably well, although some deviation from linearity is observed at 19 and 31 $\mu g \cdot L^{-1}$ in Fig. 1, presumably due to a slightly higher mortality rate in younger or smaller snails. The second assumption is that the mortality rate m can be expressed as the sum of the natural mortality m' and the lead-induced mortality m'', where m'' is proportional to the lead concentration raised to some exponent (equation 9). A theoretical analysis of biological rates, assuming these can be the product of many enzyme reactions and diffusion processes, has led to the conclusion that, in the simplest cases, rates should be proportional to substrate concentrations raised to some power, which need not be an integer (Borgmann 1974). This is consistent with our description of m''. Furthermore, the possibility of the summation of two rates to give a third (e.g. $m = m' + m''$) is also theoretically feasible (Borgmann 1974).

The above assumptions differ from those made during probit analysis, where it is assumed that the tolerance to a toxicant varies from one animal to another, with the frequency of occurrence of organisms of a given tolerance following a normal frequency distribution when plotted against log toxicant concentration (Finney 1971). If, instead of switching assumptions, we wish to calculate LC50s using the same assumptions that have proven useful in the above analysis, an alternative to the log-probit plot must be used. This can readily be derived from equations 2 and 9. Rewriting equation 2 in terms of common logarithms gives

$$(17)\quad \log N_0/N = mt(\log e) = m't(\log e) + m''t(\log e).$$

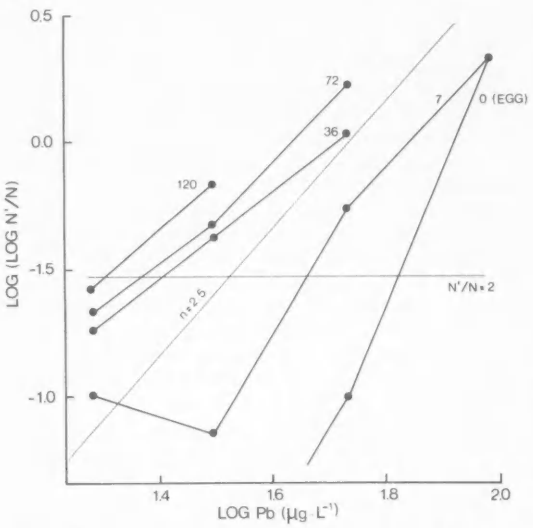

FIG. 5. Log (log N'/N), where N' is control survival and N is survival in exposed snails, plotted against log lead concentration. Numbers indicate days after hatching. A line of slope $n = 2.5$ is included and 50% survival is indicated by the horizontal line labeled $N'/N = 2$.

If N' is the number of snails surviving in the control, then

(18) $\log N_0/N' = m't(\log e)$,

(19) $\log N'/N = m''t\ (\log e)$.

Combining equations 19 and 8 gives

(20) $\log(\log N'/N) = n\log[\text{Pb}] + \log(Ct(\log e))$.

Equation 20 indicates that if $\log(\log N'/N)$ is plotted against log lead concentration, a straight line of slope n should result. The LC50 would occur at $N'/N = 2$ or $\log(\log 2) = -0.521$. Some of the data from Fig. 1 are replotted in this way in Fig. 5. A line of slope 2.5 is included for comparison. The slopes of the plots in Fig. 5 are reasonably close to 2.5, as expected from the previous estimation of n from Fig. 3. The LC50s are indicated by the intersection of these plots with the horizontal line labeled $N'/N = 2$.

Setting N'/N equal to 2 and including the values of n, C, and log e in equation 20 gives

(21) $\log[\text{Pb}] = (5.48 - \log t)/2.5$.

Equation 21 gives the LC50 as a function of time t. Using equation 21 to calculate the LC50 for the duration of the experiment (120 d after hatch plus 13 d to hatch = 133 d) gives an LC50 of about 22 $\mu g \cdot L^{-1}$. Equation 21 can be rewritten to calculate the LT50 (time to 50% mortality) for any given lead concentration.

(22) $\log t = 5.48 - 2.5 \log[\text{Pb}]$.

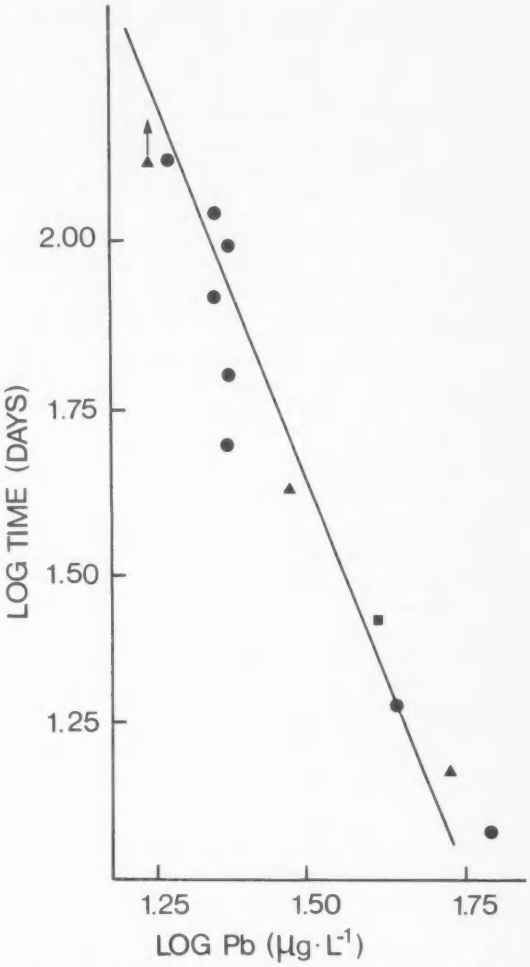

FIG. 6. The LC50 as a function of time (●) and the LT50 as a function of lead concentration (▲). The LC50 at 2 wk after hatching for a second experiment using eggs from snails collected in June 1977 is also included (■). A line of slope 2.5 is drawn through the points.

Hence a plot of log LT50 against log lead concentration should give a straight line of slope 2.5. In Fig. 6 the LC50s are plotted against exposure time and the LT50s are plotted against lead concentration on a double log plot. LC50s were obtained from the intersection of plots such as Fig. 5 with log(log 2), and the LT50s were obtained from the time when mortality was 50% of control using the solid lines in Fig. 1. Exposure time was equal to the age of the snails (days after hatching) plus the time to hatch (13 d), since egg masses were exposed immediately after being laid. The line in Fig. 6 was obtained from equation 22 and again, as in Fig. 3 and 5, the data fall approximately on the line of slope

2.5. Hence, the exponent n, to which the lead concentration must be raised to give the lead induced mortality (m''), can be obtained from direct calculation of m'', or from the plot of $\log(\log N'/N)$ against log lead concentration at any one time, or from the relationship between the LC50 and time or the LT50 and lead concentration.

Figures 5 and 6 present no new data and are merely alternative representations of the data in Fig. 1 and 3. Figure 3 is the most useful representation of the data, since m, g, and most important of all, r are all shown. This permits comparison with data from other organisms or other toxicants, in which growth, and hence production, might be reduced at concentrations below those causing direct mortality (i.e. g may be affected at lower concentrations than m).

Plots of log LT50 against log toxicant concentration, similar to Fig. 6, have been used in fish toxicology. However, the log LT50 has often been found not to be a linear function of log toxicant concentration. In many cases, these plots result in curves that are concave upwards such that at low toxicant concentrations the curve becomes parallel to the time axis and a concentration is observed below which no LT50 can be calculated. This is called the incipient LC50 and has been recommended as the most useful single criterion of toxicity (Sprague 1969). An incipient LC50 cannot be calculated for lead toxicity to *L. palustris*, since the data in Fig. 6 fall roughly on a straight line. In this snail, mortality at low lead levels continues throughout the exposure period, even after the time required for the snails to reach reproductive maturity. Even if a curve were observed in Fig. 6, this could not be considered analogous to many fish studies because of the time period involved and the rate of growth of the snails. The points in Fig. 6 at high lead concentrations (short exposure time) were obtained from snails that were very young, whereas those at low lead levels (long exposure time) are from snails that have reached maturity during the exposure time. Differences in sensitivity to lead throughout the life cycle could produce irregularities in a plot of log LT50 against log toxicant concentration if the exposure time represents a major portion of the life cycle.

Very little data on the effect of lead on long-term survival and growth is available for freshwater invertebrates. Biesinger and Christensen (1972), using Lake Superior water of hardness 45.3 $mg \cdot L^{-1}$, obtained a 3-wk LC50 of 300 $\mu g \cdot L^{-1}$ and found a 16% reproductive impairment at 30 $\mu g \cdot L^{-1}$ for *Daphnia magna*. This is higher than the lowest concentration having a toxic effect in the present study, even though the snails were exposed in harder water (139 $mg \cdot L^{-1}$). This may be due to the fact that *D. magna* is a crustacean and not a mollusc, since long-term experiments in our laboratory, using water of the same hardness as for the snails, showed no effect on growth or survival of crayfish at up to 100 $\mu g \cdot L^{-1}$ of lead (unpublished data).

Davies et al. (1976) observed growth abnormalities in rainbow trout (*Salmo gairdneri*) after chronic exposure to lead. Black tails and curved spines appeared at concentrations as low as 380 $\mu g \cdot L^{-1}$ (total lead) in hard water (353 $mg \cdot L^{-1}$) for fish exposed as fingerlings, at 14.6 $\mu g \cdot L^{-1}$ in soft water (28 $mg \cdot L^{-1}$) for fish exposed after hatching, and at 7.6 $\mu g \cdot L^{-1}$ in soft water for fish exposed from the eyed egg stage. The objective for lead proposed by the International Joint Commission (1976) and based on data for rainbow trout (Davies et al. 1976) linearily interpolated to a hardness equivalent to that of Lake Ontario water is 25 $\mu g \cdot L^{-1}$. This concentration, however, is higher than the lowest concentration observed to have a toxic effect on *L. palustris* (i.e. 19 $\mu g \cdot L^{-1}$) and causes a 20% reduction in the rate of biomass production in this snail.

Acknowledgments

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Circulation in an Open Bay

BRIAN PETRIE

Department of Fisheries and the Environment, Atlantic Oceanographic Laboratory, Bedford Institute of Oceanography, Dartmouth, N.S. B2Y 4A2

AND KEN DRINKWATER

Department of Fisheries and the Environment, Marine Ecology Laboratory, Bedford Institute of Oceanography, Dartmouth, N.S. B2Y 4A2

PETRIE, B., AND K. DRINKWATER. 1978. Circulation in an open bay. J. Fish. Res. Board Can. 35: 1116–1123.

The circulation of St. Georges Bay, Nova Scotia, under stratified conditions is presented. The mean surface circulation is characterized by a clockwise eddy with velocities of about 10 $cm \cdot s^{-1}$. Bottom (30 m) flow at the mouth is found to be <2 $cm \cdot s^{-1}$ out of the bay. Depth-averaged currents still exhibit the clockwise eddy. Although energy is found to be equally partitioned among the mean circulation, the tides, and low-frequency events, the latter determines the rate of exchange between the bay and the Gulf of St. Lawrence. Estimates of flushing time for tides and the mean circulation is of the order of a month, while low-frequency events could flush the bay on a time scale of days. Low correlation between current meters reveals the vertical and horizontal length scales of the bay are <10 m and 5 km, respectively. The circulation and exchange rates can significantly affect the dispersal (or exchanges with populations existing outside the bay) for passive particles such as fish eggs or larvae.

Key words: coastal, bay, circulation, gyre, tides, low-frequency stratified, exchange, fish larvae

PETRIE, B., AND K. DRINKWATER. 1978. Circulation in an open bay. J. Fish. Res. Board Can. 35: 1116–1123.

Nous décrivons la circulation dans la baie St-Georges, Nouvelle-Écosse, dans des conditions stratifiées. La circulation moyenne de surface est caractérisée par un tourbillon dans le sens des aiguilles d'une montre, avec vitesses d'environ 10 $cm \cdot s^{-1}$. L'écoulement de fond (30 m) à l'entrée de la baie est de <2 $cm \cdot s^{-1}$ vers l'extérieur. La moyenne des courants de diverses profondeurs montre toujours le tourbillon dans le sens des aiguilles d'une montre. Bien que l'énergie soit également partagée entre la circulation moyenne, les marées et les événements de basse fréquence, ce dernier facteur détermine le taux d'échange entre la baie et le golfe Saint-Laurent. On estime à environ 1 mo le temps de vidange pour les marées et la circulation moyenne, alors que les événements de basse fréquence peuvent réduire ce temps à une question de jours. La faible corrélation entre les courantomètres indique que les échelles de longueurs verticale et horizontale de la baie sont <10 m et 5 km respectivement. Les taux de circulation et d'échange peuvent affecter de façon significative la dispersion (ou les échanges avec des populations en dehors de la baie) des particules passives tels les oeufs et les larves de poissons.

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IN 1973 the Marine Ecology Laboratory of the Bedford Institute of Oceanography began an investigation in St. Georges Bay, N.S., of factors influencing the abundance, mortality, and growth rates of fish eggs and larvae. During 1974 and 1975 physical oceanographic measurements were included as part of the study. Two of the objectives of this measurement program were to determine the circulation in the bay and to estimate the exchange rates of water through the mouth of the bay. As the species of fish under investigation produce planktonic eggs and larvae, knowledge of the circulation may help to interpret egg and larvae distributions. Exchange rates of water will allow, to a first approximation, estimates of egg or larvae exchange between the bay and outside. This determines the biological time scale of fish eggs and larvae in the bay, i.e. the time period over which biological surveys are resampling the same egg–larval mass. Although much of the analyses are still underway, certain features of the circulation as well as some of their possible biological consequences are presented here. Complete data sets of the physical oceanography were presented by Petrie and Drinkwater (1977a, b).

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FIG. 1. St. Georges Bay, N.S. Current meter moorings were at Stations 1–8.

Study Area and Data Base

St. Georges Bay (Fig. 1) is approximately 30 × 30 km and has a mean depth of 30 m with a maximum depth of 40 m at its mouth. The bay is open on the north side to Northumberland Strait and the Gulf of St. Lawrence. Freshwater input through land drainage is small with an annual average of 57 $m^3 \cdot s^{-1}$ and a maximum mean monthly flow of 114 $m^3 \cdot s^{-1}$ (Petrie and Drinkwater 1977a).

Current meters were moored at the locations shown in Fig. 1. Sites 1, 2, and 3 were occupied in both years of the program, while moorings 4, 5, and 6 were set in 1974, and 7 and 8 in 1975. A summary of the mooring periods, instrument depths, and data return is given in Fig. 2. In 1974 the actual data return was 49% of the potential return because of instrument failure, lost moorings, and biological fouling. Instrument failure and fouling reduced the data return to 80% of the potential return in 1975. Braincon 381 film recording current meters were used only for the mooring period June–July 1974 at site 1. Aanderaa instruments, sampling rate, direction, and in most cases temperature and conductivity, were employed for all other moorings and time periods.

Bathythermograph sections were taken at weekly intervals from May to October 1974 with the main concentration at the mouth of the bay. In 1975 an Industrial Instruments RS5-3 was used to sample temperature and salinity.

Meteorological measurements were available until August 1, 1975, from an Atmospheric Environment Service station located on Port Hood Island. A tide gauge was maintained at the northern end of the Canso Causeway during 1974 and 1975.

Results

Temperature and Salinity Conditions

During the 2 yr of the program in excess of 70 sections mapping temperature alone or with salinity were taken in St. Georges Bay. One representative section is shown in Fig. 3. No measurements were made outside the bay; however, historical records (Lauzier et al. 1957) indicate that this area may be representative of the southeast portion of the Magdalen Shallows. St. Georges Bay remained stratified throughout the mooring periods in both years. When measurements began in mid-June of 1974 there was an isothermal surface layer of thickness 5–10 m and the temperature contrast be-

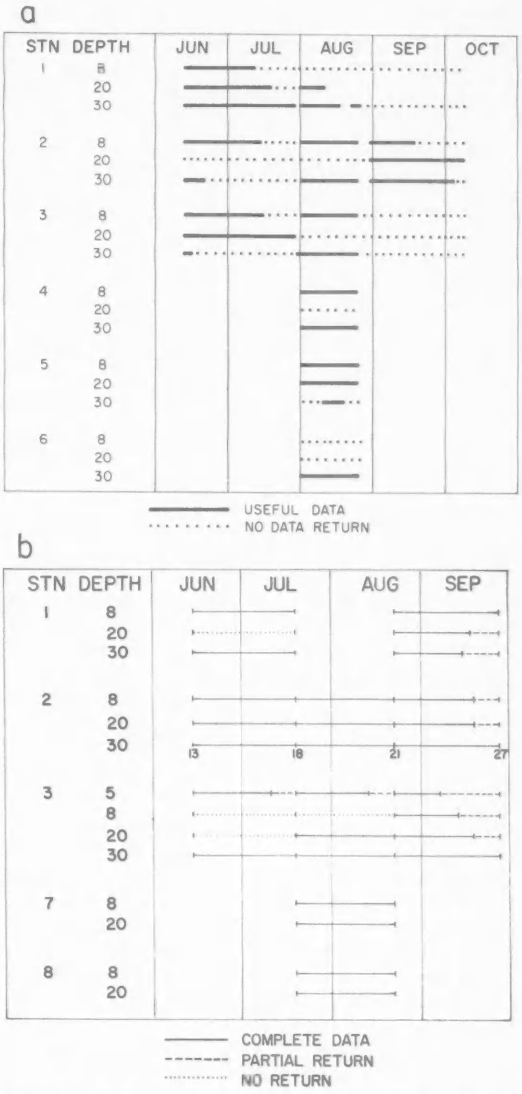

FIG. 2. a, Current meter performance record for 1974; b, current meter performance record for 1975.

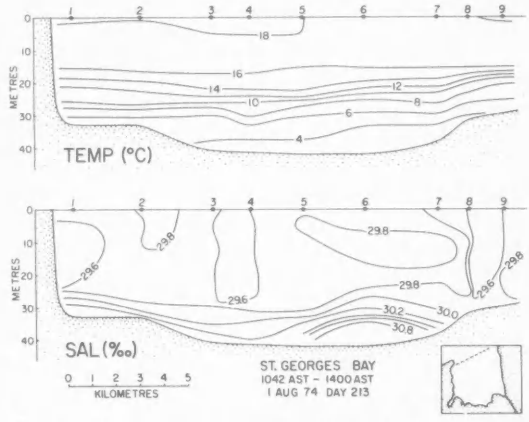

FIG. 3. Representative oceanographic section for temperature and salinity at various depths taken across the mouth of the bay on August 1, 1974.

tween surface and bottom was 8°C. By mid-July the layer was 20 m thick with a difference of 12°C. Towards the experiment's end in mid-September the layer was 30 m thick and the contrast was 10°C. Salinity at the top and bottom differed by about 1‰ throughout the summer. A similar pattern of gradual deepening of the warm surface layer was followed in 1975 but the difference between the surface and bottom temperatures was 17–19°C in mid-July 1975, considerably greater than 1974 values. The effect of the different warming rates on Atlantic mackerel (*Scomber scombrus*) spawning has been discussed by Ware (1977).

CIRCULATION IN THE BAY

For this presentation, the circulation has been divided into four categories: mean currents, low frequency, tides, and high frequency. The mean currents were defined over the length of a mooring period (about 30 d), low frequency consists of spectral bands of < 1 cycle/d (including periods of up to 8 d), the tidal band includes periods of 1 to $\frac{1}{2}$ d, and the high frequency is all the rest (12 h to 20 min).

The relative strength of the four bands is shown in Table 1. Mean currents and spectra have been calculated for all current meter records. The average root mean square amplitudes in each band are presented. In both years the tidal band is the most energetic. The mean currents and low-frequency signals are slightly smaller and about equivalent, while the high-frequency band is smaller still. The difference between the tidal and high-frequency bands is significant at the 95% confidence level. These bands are discussed separately below with particular reference to the rate at which water and consequently fish eggs and larvae could be exchanged between the bay and the Gulf of St. Lawrence. In addition, their effect on the distribution of biological material within the bay is discussed.

TABLE 1. Average root mean square amplitudes (cm/s).

	Mean	Low	Tides	High
1974	6.5	6.8	8.2	4.5
1975	5.3	7.7	9.0	4.4

TIDAL AND HIGH-FREQUENCY BANDS

The largest tidal constituent in St. Georges Bay is the M_2 component having currents normal to the mouth of about 10 cm·s^{-1} and a tidal elevation at Canso of 24.4 cm. The flows associated with the other major constituents K_1, O_1, and S_2 are about 1–3 cm·s^{-1}. The exchange rate of bay water was estimated for the case of complete mixing of the tidal volume and depended on the tide height and average bay depth. An estimate was also made for the advection away of the tidal volume and was a function of the horizontal tidal excursion at the bay mouth, the area of the bay, and the strength of the external current. If, on each cycle, the tidal volume were mixed completely with water in the bay, then the e-folding time for removal of original bay water would be about 15 d. But, as indicated earlier, the bay is highly stratified throughout the summer, therefore complete vertical mixing did not occur. Moreover, the horizontal tidal excursion into the bay is about 1 km; thus horizontal mixing throughout the bay is unlikely. The 15-d estimate is regarded as a lower bound and a realistic exchange rate by this process would probably be several times this value. For the advective case, a tidal volume moving outside the bay would be partially swept away by an external current. An eastward setting current of 10 cm·s^{-1} was chosen (Farquharson 1959; Lauzier 1965) and it was assumed that only original bay water was lost on a tidal cycle until the system was flushed completely. The e-folding time was 140 d. From these estimates we expect tidal processes to flush the bay on time scales of the order of a month. To be reasonably sure of sampling a statistically identical population, biological studies carried out in the bay should be sampled more rapidly than once per month. In addition, to assure continuity from one measurement to another in biological studies such as on growth and mortality, the processes should have a time scale of the order of 1 mo or less.

The high-frequency spectral estimates had the least amount of energy and were incoherent vertically and horizontally between current meters. A rather crude estimate of their effect on flushing the bay may be made in the manner of Garrett and Munk (1972) by assuming all of the energy in the high-frequency band goes into mixing. One arrives at an e-folding time for diffusion of water out of the bay of 700 d. Clearly the high-frequency band does not appear to be important to flushing.

LOW-FREQUENCY BAND

Although there was a significant amount of energy in

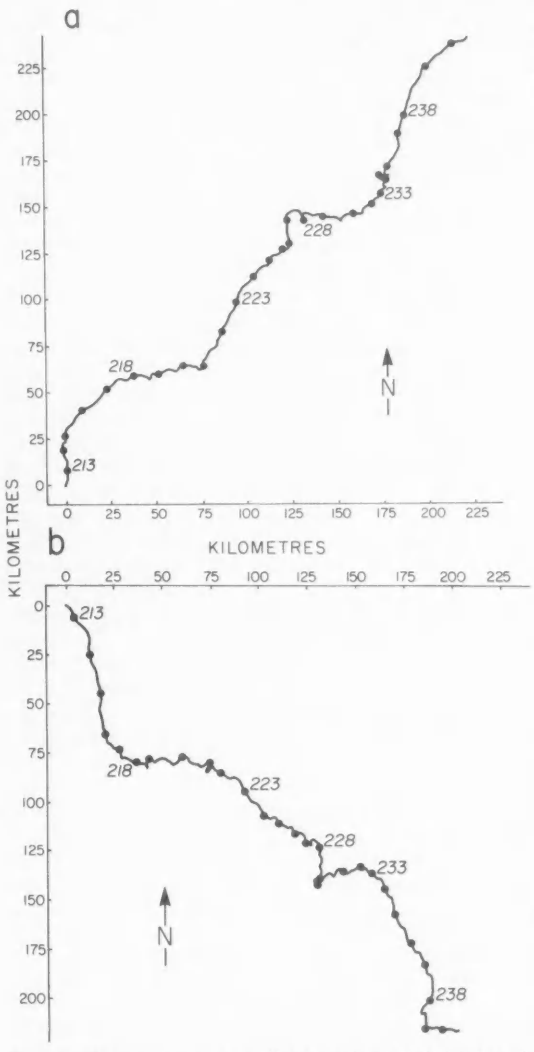

FIG. 4. Progressive vector diagrams from (a) Station 4 and (b) Station 2, 8 m, for the period July–August 1974. The current moves to the southeast at Station 2 and to the northeast at Station 4.

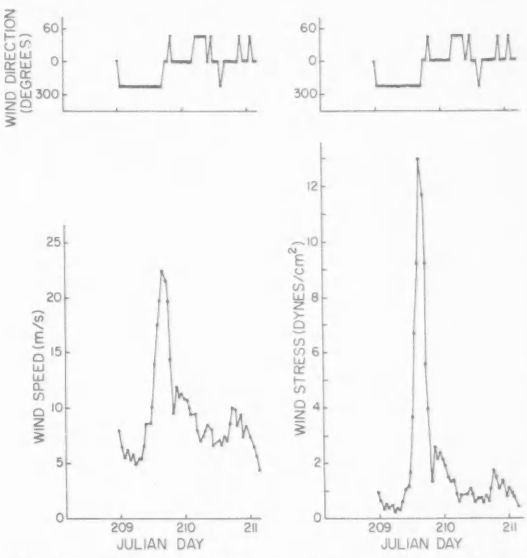

FIG. 5. Wind and wind stress (calculated according to Smith and Banke [1975] for July 28–29 (Julian days 209–210), 1975. Wind direction is direction wind is blowing towards.

the low-frequency band, in general, this variability was not coherent either vertically or horizontally. This was true for both years despite the fact that instruments were separated by only 10 m vertically and as close as 5 km horizontally. The only frequency band for which the coherence was consistently above the 95% confidence limit was the tidal band. A mere 15% of the possible vertical pairs (like components of current resolved into eastward and northward directions) gave values of coherence above the 95% confidence limit for the 1975 data. The figure was 10% for horizontal pairs (both like and cross components) at the same depths. Simple correlations were also tried on the 1974 data with identical results. The data had been filtered to remove tides and in some cases rotated to bring preferred current directions into alignment. The currents at Stations 2 and 4, 8 m, are plotted as progressive vector diagrams in Fig. 4. The overall trends of the data were similar and low-frequency features in one plot appear to be mirrored in the other. The correlations which were calculated for the components normal and parallel to the mouth of the bay were expected to be large. The largest value, for the normal component, was −0.49 accounting for only 25% of the variance. One progressive vector diagram was rotated to align with the preferred direction of the other and longitudinal and transverse correlations were calculated with no significant improvement. A complete set of coefficients is given by Petrie and Drinkwater (1977a). The lack of coherence and the significant energy content of the low-frequency band imply that the length scale or patch size of any biological material carried passively by the flow would be < 5 km.

The average low frequency spectrum from data taken at the mouth of the bay would give, on the basis of complete mixing, a flushing time of 10 d. However, this estimate has little validity, since the bay was not well mixed and there was little coherence between sensors in the bay. Therefore, it is difficult to assign a time scale for flushing due to the low-frequency portion of the spectrum. Rather, events, preferably ones with large amplitudes, must be examined so some bound may be

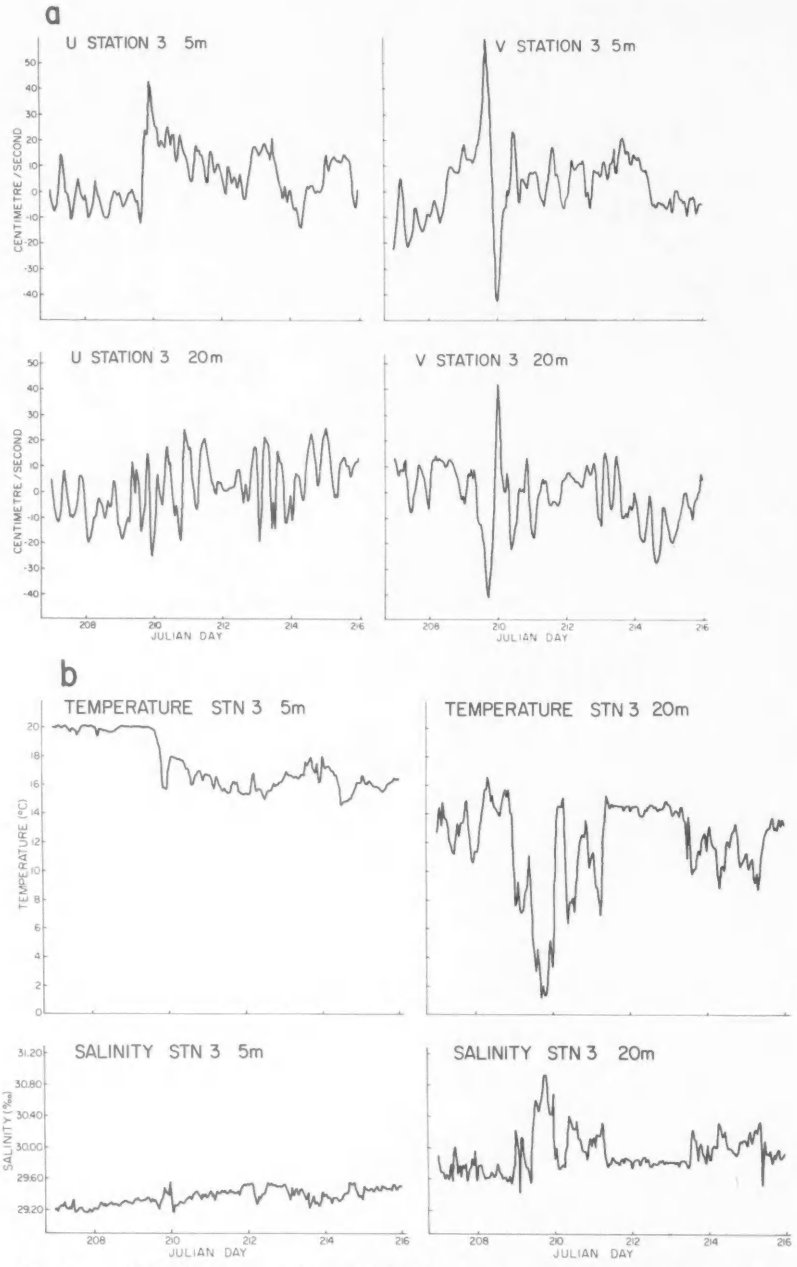

FIG. 6. a, *U* (positive eastward) and *V* (positive northward) currents at Station 3, 5 and 20 m, during the storm; b, temperature and salinity at Station 3 during the storm.

placed on flushing times. The most exceptional event in the 2 yr occurred on July 28–29 (Julian days 209–210), 1975. It is also the exception to the discussion above, since it was coherent in all records taken in the bay at that time. During this period winds up to 23 $m \cdot s^{-1}$ (Fig. 5) blew from the south and were in excess of 10 $m \cdot s^{-1}$

for 15 h. Moorings 2, 3, 7, and 8 were operating at this time. The response was similar at all moorings and is illustrated for Station 3 in Fig. 6.

Consider the V (positive to the north) component first. At the 5 m depth water was forced out of the bay with velocities up to 60 $cm \cdot s^{-1}$ with the buildup of the storm. This caused a depression of the water level by some 5 cm as measured at the Canso Causeway. The maximum lowering of water level corresponded to the peak of the storm. As the winds weakened, sea level rose above its mean by about 3 cm and the flow reversed, rushing back into the bay with speeds up to 45 $cm \cdot s^{-1}$. At 20 m, the V component was 180° out of phase with that at 5 m, reaching speeds of 45 $cm \cdot s^{-1}$. These records and others indicated that inertial period motion continued for 2 d after the storm ended. The flow at 30 m behaved in a similar fashion to that at 20 m. Temperature (Fig. 6b) at the upper depth was lowered by some 4°C with the inflow and never regained its former level. This may have been due to mixing induced by the storm or an exchange with cooler waters outside the bay. Salinity was less affected. Temperature and salinity at 20 m showed pronounced effects and inertial oscillations, maybe supplemented by tides, which persisted. Station 2, at the eastern side of the mouth of the bay, responded in a similar way as did the current meters on moorings 7 and 8 within the bay. The event then was coherent and in the surface layers moved 18- and 12-km-long slabs of water out of the bay at Stations 3 and 2, respectively. Now certainly some of this was returned on the incoming flow, but as an upper bound roughly 30–50% of the water was forced out of the bay and potentially exchanged. The data of Harding et al. (1978) give an indication of the effect such a storm might have on biological populations of the bay. They found that the mean density of American lobster (*Homarus americanus*) larvae of $3.8 \times 10^3 \cdot km^{-2}$ 1 wk before the storm had decreased by a factor of 2–$1.9 \times 10^3 \cdot km^{-2}$ 2 d after the event. By August 4–5 the value had recovered to $3.2 \times 10^3 \cdot km^{-2}$. The storm then did disrupt the lobster larvae and presumably other populations in the bay. Moreover, there is no way of knowing whether the populations after the storm were equivalent to those earlier nor how growth and mortality studies might be influenced. Clearly, low-frequency energetic events which can give flushing times of 2–3 d may have a profound effect upon such studies. Strong currents, though not wind driven, also occurred in 1974 and could have flushed the bay on a time scale of a few days.

We have considered a simple model of this wind event for the upper layer of St. Georges Bay. It could prove to be qualitatively useful in similar situations when current meter data were unavailable. The momentum balance was between the time derivative of velocity, the Coriolis force, and a wind-stress term that varied linearly with time. The initial increase of wind stress and subsequent decrease were considered with solutions patched together at the peak of the storm. The depth of the upper layer was taken as 15 m. At time $t = 0$, which was defined as 5 h before maximum wind stress, the initial velocities were taken as those measured by the current meter at Station 3, 5 m deep. The solution consisted of a forced part and inertial waves. While the simple model gave reasonable currents up to the peak of the storm (model, U (positive to the east) = 8 $cm \cdot s^{-1}$, $V = 61$ $cm \cdot s^{-1}$; data, $U = -4$ $cm \cdot s^{-1}$, $V = 59$ $cm \cdot s^{-1}$ at the peak) it fared poorly as the wind stress dropped. The model predicted a reversal in the V component 2 h after the peak of the storm, while the data indicated that the reversal did not occur for some 5 h. Other processes such as frictional dissipation, land boundary effects, and pressure gradients are obviously important and need to be considered in detailed model studies.

Mean Circulation

Figure 4 illustrated the persistent nature of the direction of current flow in St. Georges Bay which was true with few exceptions for all data in 1974 and 1975. For each of the three mooring periods we have constructed a diagram (Fig. 7) of the mean currents at all three depth levels as well as a depth integrated (vector mean) circulation pattern. The greatest number of measurements was taken in the July–August period (Fig. 7b) in both years.

The circulation at 8 m deep is strongly clockwise with mean currents ranging from 5.0 to 13.6 $cm \cdot s^{-1}$. This clockwise motion is mirrored somewhat at 20 m deep but is nonexistent at 30 m, where the flow is basically out of the bay. The integrated circulation is also given where at least two measurements were available from one station and one of the data sets was from 8 m deep. The anticyclonic pattern persists here also and has been used for modeling purposes. The data from the two other mooring periods are consistent with the clockwise circulation pattern.

To calculate the exchange time for the mean circulation the flows for the most heavily sampled period, July–August 1974, were decomposed into components normal and parallel to the mouth of the bay which runs along 68°T (true compass). For this period, surprisingly in view of the lack of coherence shown previously, the net fluxes balanced if the current meters were taken as representing equal areas. The turnover time would be roughly 40 d barring the possibility of recirculation. Recirculation of water is a real possibility since, at least in the upper 20 m, water leaving the bay on the western side and moving east (all records at 8 and 20 m have eastward flow) could reenter the system on the eastern side. Thus the exchange time could be longer than 40 d.

The eddy would act as a retention mechanism to keep an egg–larvae–plankton population within St. Georges Bay. Since the time scale from eggs to larvae for the species investigated by the Marine Ecology Laboratory is about 2 wk, or less than the flushing time scale of the mean circulation, the egg and larval stages would likely be spent entirely within the bay. This is barring any low-frequency event that could flush the bay or large parts of it.

FIG. 7. a, Mean currents in St. Georges Bay for the mooring period June–July 1974 and 1975. The integrated circulation is the vector mean of the data for which measurements at two levels were available; b, mean currents in St. Georges Bay for the mooring period July–August 1974 and 1975; c, mean currents in St. Georges Bay for the mooring periods August–October 1974, and August–September 1975.

What mechanism could generate this consistent pattern of clockwise circulation? As a first attempt to answer the question atmospheric forcing was investigated. However, in general, there was little correlation between winds and currents. Investigations by Lauzier (1965) and Farquharson (1959) indicated the presence

of a current flowing from west to east past St. Georges Bay and along the Cape Breton shore. Current meter measurements presented here are consistent with this flow. Consequently a barotropic model was formulated with such a current as the driving mechanism. Vorticity and continuity equations were solved numerically for a rectangular bay, which St. Georges Bay closely approximates, with natural bottom topography. Additional details of the model are given by Petrie and Drinkwater (unpublished data). With the appropriate choice of horizontal and vertical eddy coefficients a clockwise circulation pattern can be generated in the bay consistent with observations.

Acknowledgments

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Stress Response of Coho Salmon (*Oncorhynchus kisutch*) Elicited by Cadmium and Copper and Potential Use of Cortisol as an Indicator of Stress[1]

CARL B. SCHRECK

Oregon Cooperative Fishery Research Unit,[2] Oregon State University, Corvallis, Oreg. 97331, USA

AND HAROLD W. LORZ

Research Section, Oregon Department of Fish and Wildlife, 303 Extension Hall, Oregon State University, Corvallis, Oreg. 97331, USA

SCHRECK, C. B., AND H. W. LORZ. 1978. Stress response of coho salmon (*Oncorhynchus kisutch*) elicited by cadmium and copper and potential use of cortisol as an indicator of stress. J. Fish. Res. Board Can. 35: 1124–1129.

Exposure of juvenile coho salmon (*Oncorhynchus kisutch*) to copper (Cu) produced a marked, dose-dependent serum cortisol elevation. Treatment with cadmium (Cd) did not elicit a cortisol elevation, even in moribund fish. Stressing salmon with sublethal levels of Cu or handling plus close confinement resulted in "ideal" compensation (return to pre-stress levels) in cortisol titers. Salmon exposed to Cu had depressed serum chloride levels and reduced survival when challenged with salt water. Exposure to Cd did not influence serum chloride or the ability to tolerate salt water. Handling and close confinement produced the same cortisol elevation in controls as in Cu- or Cd-treated fish, but exposure to Cu reduced the ability of the fish to survive the stress of handling and confinement. Cortisol level and other characteristics of the General Adaptation Syndrome of stress should not be universally applied as indicators of stress in salmon.

Key words: stress, cortisol, heavy metals, coho salmon, *Oncorhynchus kisutch*, saltwater adaptation, stress resistance, stress indicator, cadmium, copper

SCHRECK, C. B., AND H. W. LORZ. 1978. Stress response of coho salmon (*Oncorhynchus kisutch*) elicited by cadmium and copper and potential use of cortisol as an indicator of stress. J. Fish. Res. Board Can. 35: 1124–1129.

Quand des jeunes saumons coho (*Oncorhynchus kisutch*) sont exposés à du cuivre (Cu) il y a élévation marquée, dépendante de la dose, du cortisol sérique. Un traitement au cadmium (Cd) ne provoque pas d'élévation de cortisol, même chez les poissons moribonds. Le fait de soumettre les poissons à un stress par des doses sublétales de Cu ou par manipulation et maintien dans un espace restreint résulte en une compensation « idéale » (retour aux niveaux d'avant-stress) dans les titres de cortisol. Lorsque confrontés avec de l'eau salée, les saumons exposés au Cu accusent des niveaux abaissés de chlorure sérique et une survie amoindrie. L'exposition au Cd n'affecte pas le chlorure sérique ou l'aptitude à tolérer l'eau salée. La manipulation et le maintien en espace restreint produisent la même élévation de cortisol chez les témoins que chez les poissons traités au Cu ou au Cd, mais l'exposition au Cu diminue l'aptitude des poissons à survivre au stress causé par la manipulation et le maintien en espace restreint. On ne devrait pas utiliser universellement, comme indicateurs de stress chez les saumons, le niveau de cortisol et autres caractéristiques du syndrôme général d'adaptation du stress.

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HEAVY metals are widely distributed in aquatic systems and can affect fish populations by reducing growth, reproduction, or survival. One would suspect that metals would produce stress in fish, within the context of Selye (1950, 1973), particularly when death results. Hormones, primarily the corticosteroids, are believed to mediate the stress response by serving as a stimulus between the neural transducer and other factors of the General Adaptation Syndrome which consists of the physiological responses elicited by most stressing agents (Selye 1976; Mazeaud et al. 1977; Strange et al. 1977). Secondary stress responses such as changes in plasma

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and liver sugar levels, immunosuppression, and blood cell profile are thought to be in response to elevation in corticoid hormones and catecholamines. Acute exposure of sockeye salmon (*Oncorhynchus nerka*) to copper (Cu) produced a marked, dose-dependent elevation in corticoids monitored for 24 h (Donaldson and Dye 1975). Our objectives were to determine whether heavy metals produced a general corticoid stress response in fish, and, if so, to describe the dynamics of plasma cortisol levels over time to either acclimation (compensation) or exhaustion (death) — thereby characterizing potential response curves as outlined by Precht (1958). Our goal was to determine the feasibility of using blood cortisol levels as an indicator of heavy metal toxicity.

We administered Cu and cadmium (Cd) to juvenile coho salmon (*O. kisutch*). There is no a priori way of determining whether fish are stressed by sublethal concentrations of metals before they die. We used resistance to a second stress — either severe confinement and handling or survival in salt water — as a criterion for determining whether the fish had been stressed by the metals. We correlated these data with cortisol titers and speculated on a new hypothesis concerning corticoid elaboration and the stress response in fish.

Materials and Methods

Coho salmon eggs of the 1974 and 1975 year-classes obtained from Fall Creek Salmon Hatchery (Oregon) were incubated at 8–10°C. Fry and fingerlings were reared under natural light in well water at 12.3°C and fed Oregon moist pellets daily. Fish were yearlings during the tests and were 15–22 cm long. They were not fed during acute tests. Flow-through exposures were conducted in 1000-L, 1.54 m diam tanks with a gravity flow diluter delivering 12 L/min; 95% of the water in the tanks was replaced every 3.7 h. Details of the delivery system were described by Lorz and McPherson (1977). Background levels in the well water were less than 3.2 μg/L Cu and less than 1 μg/L Cd. Chemical characteristics of the well water were alkalinity — 76 mg/L as $CaCO_3$, hardness — 90 mg/L as $CaCO_3$, dissolved oxygen — 9.5 mg/L, ammonia — 0.154 mg/L, and pH — 7.03 (data from Lorz et al. 1978). Measured (flameless atomic absorption) concentrations of Cd and Cu used in tests are presented in Table 1. These analyses were run by the Environmental Protection Agency, Western Fish Toxicology Station. The tanks were exposed to natural light as well as to a simulated natural photoperiod provided by Vita-Lite fluorescent bulbs. Tanks were screened with black polyethylene to reduce disturbances.

During sampling, fish were captured as rapidly and with as little disturbance as possible, stunned by a blow to the head, and exsanguinated by severance of the caudal peduncle. It typically took <5 min to collect samples from the 10 fish from one tank. Serum, obtained by letting the blood clot at least 15 min and then centrifuging it, was stored at −15°C until analyzed. Cortisol was assayed in 25 μL of serum by using a competitive protein binding technique proposed by Murphy (1967) and modified to eliminate extraction by Strange and Schreck (1978). Corticoids determined by this procedure are 80% cortisol. Serum osmolarity was determined with a Wescor Model 5100 vapor pressure osmometer and chlorinity with a Corning Model 920 chloride meter.

Histological sections stained with hematoxylin–eosin were made from tissues of fish exposed to either Cd or Cu.

Statistical significance is judged on overlap of 95% confidence intervals (*t*-test).

TABLE 1. Nominal and measured concentrations of cadmium and copper used in acute tests with coho salmon.

Metal and date	Nominal (μg/L)	Measured concn (μg/L) Mean ± SE		*n*
Cadmium	0	0.27	0.09	12
June 21–July 2, 1976	4	4.46	0.28	12
	8	8.13	0.24	12
	12	10.67	0.21	12
Copper	0	0.8	0.30	5
Dec. 27–Jan. 6, 1977	15	13.8	0.13	5
	60	52.8	1.00	6
	90	76.1	0.75	6
Copper	0	1.37	0.43	3
Jan. 11–Jan. 18, 1977	140	94.1	11.04	4
	210	162.0	9.82	4

Experimental Design and Results

CADMIUM — CHRONIC TESTS

To determine sublethal effects of long-term exposure to Cd, we treated five replicated groups of about 350–420 fish each continuously, starting on February 26, 1976, with 0, 0.75, 1.5, 3.0, or 4.5 μg/L of Cd (as $CdCl_2$). After 48-d chronic exposure, on April 14, 1976, 15 fish were sampled from each of the 0 and 4.5 μg/L groups. Cortisol levels of the control and Cd-treated fish were not significantly different (Fig. 1).

We sampled fish at 0900 67 d after onset of the 0, 0.75, 1.5, 3.0, and 4.5 μg/L treatments. Cortisol levels did not differ between fish in the various treatments or from the concentrations found 1 mo earlier (Fig. 1).

We also suspended 10 fish each from the 0 and 4.5 μg/L groups in a 25- × 40-cm dip net immersed in a bucket of water from their original tanks to determine whether the Cd-treated fish elaborated cortisol due to this type of handling stress, as reported by Strange et al. (1977). Capture and 15-min confinement produced a fourfold elevation in cortisol titers in both the control and Cd-treated fish (Fig. 1).

To see if Cd-exposure impaired the ability to adapt to seawater, we transferred 10 fish each from the 0 and 4.5 μg/L tanks to 0.61 m diam fiberglass tanks containing 120 L natural seawater (30‰ salinity) maintained at 10°C under simulated natural photoperiod. For controls, we transferred 10 fish each from the 0 and 4.5 μg/L groups to similar tanks containing water from their original tanks, and also transferred fish from the 4.5 μg/L treatment to a tank containing freshwater but

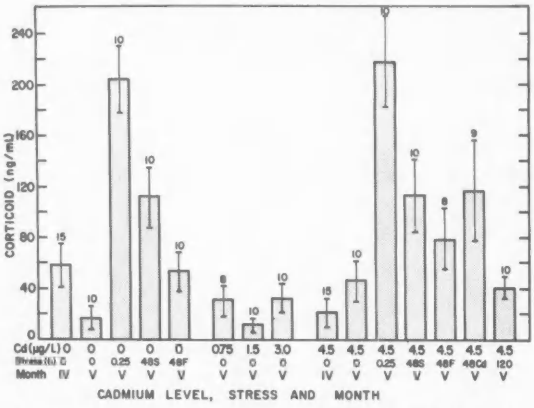

FIG. 1. Mean cortisol (corticoid) levels (±SE ⌶) in coho salmon exposed to 0, 0.75, 1.5, 3.0, and 4.5 μg/L cadmium since February 26, 1976 and sampled during April (IV) or May (V) (stress = 0) and after being (1) confined 0.25 h in a dip net suspended in a bucket (stress = 0.25), (2) transferred for 48 h to a smaller tank with static salt water (stress = 48S) or freshwater (stress = 48F) or water with cadmium from their original tank (stress = 48Cd), or (3) transferred for 120 h to a smaller tank with freshwater and no cadmium (stress = 120).

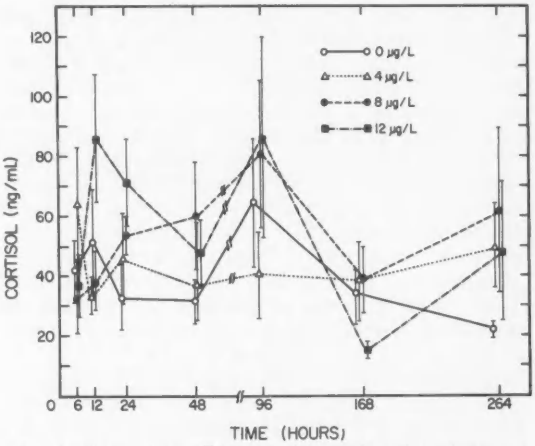

FIG. 2. Mean (±SE ⌶) cortisol levels in coho salmon exposed to various levels of cadmium for different lengths of time.

no Cd. After 48 h, mean cortisol levels of saltwater-challenged fish and those transferred to freshwater in both the 0 and 4.5 μg/L groups were similar and appeared intermediate between "basal" levels and those encountered after 15 min of stress (Fig. 1). The Cd-treated fish were fully able to adapt to seawater. However, these "intermediate" values did not differ significantly from "basal" levels.

We placed 10 fish from the 4.5 μg/L treatment into a 0.61-m tank supplied with flowing water but no Cd. These fish were sampled 120 h after transfer; mean cortisol levels had completely returned to basal values within this time after handling (Fig. 1).

Fish exposed to 4.5 μg/L Cd for 70 d did not differ histologically from controls in gonad, liver, head and tail kidney, and gill structure.

CADMIUM — ACUTE TESTS

To determine whether lethal levels of Cd affect cortisol level, we acclimated 35–47 coho salmon for 7 d in each of eight tanks. On June 21, 1976 duplicate tanks of fish were exposed starting at 0730 to 0, 4, 8, or 12 μg/L Cd as $CdCl_2$. Samples of fish were taken from alternate tanks of the same dosage at each sampling period to minimize stress due to disturbance. Ten fish were sampled from a tank at each dosage at 6, 12, 24, 48, 96, 168, and 264 h after the start of treatment.

No mortality occurred in the control or 4 μg/L groups. The average mortality for the 8 and 12 μg/L treatments through 144 h were 14.3 and 53.1%, respectively. The fish never behaved violently in response to the toxicant; instead, they appeared sedated before they died. No cortisol response was evident, even during periods of high mortality (Fig. 2). Osmolarity and chlorinity of the serum did not vary with treatment or duration of exposure (Lorz et al. 1978). Histological examination of fish of the 12 μg/L group revealed no discernible differences from controls after 120 h of Cd exposure.

COPPER — ACUTE TESTS

Sublethal and lethal concentrations of Cu were used to indicate whether there is a general lack of cortisol secretion due to heavy metal stress as indicated with Cd.

For sublethal tests, 80–144 yearling coho salmon per tank were acclimated for 7 d. The fish were exposed to 0, 15, 60, and 90 μg/L Cu (as $CuCl_2$) starting at 0730 on December 27, 1976. Samples of 10 fish were taken from each group at 2, 8, 24, 36, 78, and 170 h thereafter. No deaths occurred over the 170-h period of Cu treatment. However, toward the end of this exposure period, the fish in the 60- and 90-μg/L Cu concentrations appeared distressed; we suspect that some mortality would have occurred eventually. There was an immediate, dose-dependent increase in serum cortisol after Cu exposure began (Fig. 3A), followed by a return to more basal levels within 8–24 h. Thereafter, the cortisol titers of fish at the higher concentrations increased significantly and remained elevated until the end of the experiment.

We used resistance to a second stress as an index of reduced fitness. Two groups of 19 fish each were taken at 170 h from both the control and 90 μg/L treatment and placed in 17 cm diam perforated buckets immersed 8 cm in tanks with water similar to that in the experimental tanks. This process subjected the fish to severe stress involving handling and confinement. Blood from

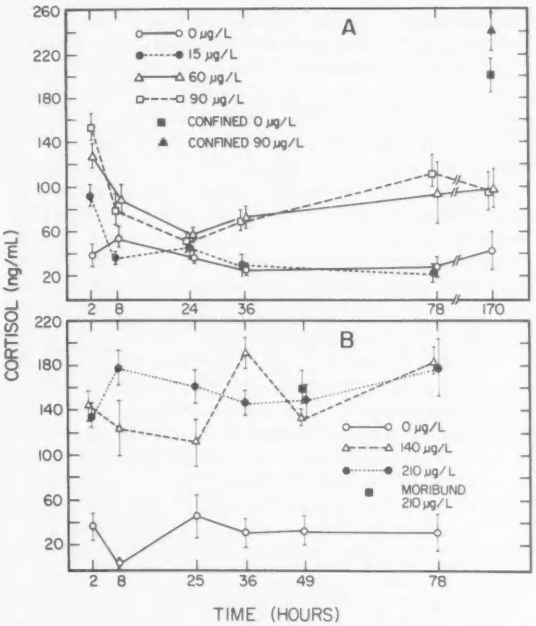

FIG. 3. Mean (±SE ⌶) cortisol levels in coho salmon exposed to (A) sublethal levels of copper for various lengths of time or subjected to confinement in a small live cage after exposure to 0 or 90 µg/L of copper, or (B) control or lethal levels of copper; square represents mean of fish sampled just before death.

the fish in one bucket from each treatment was sampled 1 h after confinement. Mortality was monitored for fish in the other buckets. Fish from each treatment were also transferred to tanks containing 120 L natural seawater. Fish from the 0 and 90 µg/L treatments confined for 1 h in the buckets exhibited similar and greatly increased cortisol levels due to the stress. Survival during confinement, however, was markedly different between the two groups. Fish receiving the Cu had a median survival time of about 12–15 h while the control group experienced no mortality even after 36 h of confinement. There was a dose-dependent relationship between Cu level and survival in seawater with the fish exposed to higher levels of Cu not able to adapt to the 30‰ salinity (Table 2). Survivorship in salt water appeared to be inversely correlated to cortisol level.

To determine responses to lethal concentrations, we acclimated 110 yearling coho salmon per tank for 7 d before treatment with 0, 140, or 210 µg/L Cu as $CuCl_2$. Ten fish from each group were sampled at 2, 8, 25, 36, 49, and 78 h after onset of treatment. Also, three moribund fish were sampled after 49-h exposure to 210 µg/L to determine whether ensuing death altered cortisol levels. The median survival times were 60 and 48 h for the 140 and 210 µg/L groups, respectively. Exposure to 140 and 210 µg/L Cu resulted in similar, immediate elevations in cortisol levels (Fig. 3B). The

TABLE 2. Survival of yearling coho salmon exposed to copper in freshwater and their later survival after transfer to seawater (without copper).

Nominal Cu concn (µg/L)	Number of fish	Survival (%) after 190-h exposure	
		Freshwater	Seawater
Control	14	100	100
15	20	100	60
60	23	100	0[a]
90	18	100	0[a]

[a]All fish died after seawater exposures between 40 and 96 h.

TABLE 3. Serum osmolarity and chlorinity of coho salmon exposed to copper for various lengths of time (sample size, 5). SE in parentheses.

Exposure concn	Duration of exposure (h)	Osmolarity (mosmol)	Chlorinity (mEq/L)
Control	2	284.4 (1.176)	112.9 (1.26)
	8	286.6 (3.24)	120.0 (2.78)
	26	292.6 (1.69)	114.6 (0.53)
	36	295.9 (1.61)	114.0 (1.79)
	49	291.6 (2.19)	115.3 (1.69)
	78	285.6 (3.78)	113.4 (1.05)
140 µg/L	2	296.8 (4.25)	116.4 (1.07)
	8	292.9 (1.91)	111.9 (1.37)
	26	292.8 (5.19)	110.9 (1.37)
	36	292.6 (2.75)	94.4 (1.66)
	49	286.7 (2.93)	88.1 (1.58)
	78	293.5 (2.33)	84.5 (2.24)

increases in cortisol were similar to those among fish treated with 60 and 90 µg/L Cu (Fig. 3A). At 140 and 210 µg/L Cu, the cortisol levels remained high throughout the test period and did not differ from that of the three moribund fish. Cortisol levels in Cu-exposed fish were as high as titers produced by severe handling and confinement stresses as seen in Fig. 1 and 3A. Exposure to 140 µg/L produced no change in serum osmolarity, but chloride level decreased significantly after 26-h exposure (Table 3). Histological examination of controls and salmon exposed to 210 µg/L Cu for 25 or 49 h revealed normal livers in both groups. Kidneys of Cu-exposed fish had glomerular atrophy, cells with condensed pyknotic nuclei, and depletion of hematopoietic tissue. Gills of the treated fish exhibited slight epithelial necrosis and some hyperplasia and fusing of adjacent lamellae.

Discussion

The two metals appear to have completely different modes of action in eliciting a general stress response in coho salmon. Exposure to Cd caused death of yearling salmon without elevating their cortisol levels, while Cu exposure was followed by a large, dose-dependent cortisol response. We found in preliminary study that con-

current exposure of salmon to Cd plus Cu resulted in a cortisol response typical for the Cu component of the mixture. Acute exposure to mercury also appears to elevate cortisol (Lorz et al. 1978). The use of cortisol (and probably other clinical–chemical characteristics controlled by this steroid) as an index of the "health" of salmon thus depends on (1) the specific nature of the stress, (2) the severity (or concentration) of the stress, and (3) the length of time that the fish have been exposed to the stress. Hence, clinical chemistries such as cortisol determinations probably are not a good indicator of the general health of fish, for "normal" titers do not negate the possibility that fish are severely stressed.

Evaluation of resistance to a second stress is a useful means of assessing sublethal effects of the first stress. In addition, increased cortisol levels can be correlated with reduced resistance. For example, sublethal challenges of salmon with Cu reduced survival after confinement, and cortisol levels increased substantially. After application of Cu, the yearling salmon were capable of maintaining elevated cortisol levels for up to 170 h without evidence of interrenal exhaustion.

Final levels of serum cortisol were the same between coho salmon merely stressed by confinement and those confined after cortisol titers had been substantially raised as a result of exposure to Cu. Also, acclimation to 4.5 μg/L Cd did not influence the dynamics of the stress response to another stress (handling). Complete compensation (return of cortisol to normal levels) following transfer of fish from one tank to a smaller tank required 48–120 h.

We attribute the different cortisol reactions between Cu- and Cd-treated fish to the possibility that Cu is more noxious than Cd. Adult Atlantic salmon (*Salmo salar*) actively avoided Cu (Sprague et al. 1965). We noticed that fish treated with Cu became restless and appeared to "fight" the toxicant, whereas Cd-exposed coho salmon simply appeared to be narcotized before death. Other investigators, however, noted hypersensitivity to external stimuli, often resulting in violent swimming movement at high concentrations of Cd in bluegill (*Lepomis macrochirus*) (Eaton 1974) and rainbow trout (*Salmo gairdneri*) (Kumada et al. 1973; White 1977). Cd at levels used here did not affect serum chloride levels, gill Na^+-K^+ ATPase, histology, saltwater adaptation, or downstream migration of smolts (Lorz et al. 1978), whereas Cu had a direct effect on all of these factors (Lorz and McPherson 1976, 1977). Elevated cortisol level is probably related either to creating the abnormal chloride and ATPase levels or to the process of trying to restore the values to normal, since corticoids have been implicated in electrolyte balance and gill ATPase activity (Epstein et al. 1971; Utida et al. 1972; Johnson 1973).

The dynamics of circulating cortisol levels in Cu-exposed fish depends on the concentration of the metal. The alarm phase of stress (Selye 1950) is characterized by an immediate increase in cortisol at all levels of Cu tested, albeit not for Cd-exposed fish. Similar results were noted in Cu-treated fish by Donaldson and Dye (1975). We presume that this early elevation of cortisol by Cu reflects the reaction of the fish to the recognition of the presence of a noxious or potentially harmful substance. This response lasted a few hours and was followed by the resistance phase. Cortisol levels returned almost to basal levels within 24 h at Cu concentrations up to 90 μg/L. Resistance is the phase of the stress response following the initial reaction (alarm) to the stressing agent and during which the organism tries to maintain or regain homeostatis. Resistance led to compensation at 15 μg/L Cu but to further resistance or perhaps eventual exhaustion (death) at 60 or 90 μg/L as seen by the second, gradual elevation in cortisol titers throughout the rest of the test period. The alarm, resistance, and exhaustion phases could not be readily distinguished at Cu concentrations of 140 μg/L Cu or above, for cortisol levels remained elevated and stable throughout the period of exposure, even at death. Although cortisol levels were increased in fish made moribund by Cu exposure, mean titers never reached those exhibited by handled and confined fish. If the fish were able to acclimate to the Cu (e.g. at 15 μg/L), their cortisol pattern followed a Precht (1958) type 2 shape, yielding "ideal" compensation. This pattern indicated that although the stress (Cu) was still present at the same severity, the cortisol levels of the fish returned to prestress conditions.

We believe, consequently, that some but not all stresses produce elevations of cortisol, and hence the mammalian concept of the General Adaptation Syndrome may not directly apply to fish. In anthropomorphic terms, it appears to us that stimuli that produce fright, discomfort, or pain in the fish elicit the General Adaptation Syndrome-type reactions. The practicality of using cortisol levels as a general index of environmental quality or fish health is thus limited. The variable response patterns and the fact that sublethal concentrations of a toxicant can result in "ideal" compensation further complicate this matter. Also, some toxicants (e.g. Cd) can be highly lethal yet produce no cortisol reaction. We suggest that physiological studies directed toward assessment of fish health or water quality should include determinations of response curves for various concentrations of the factors of interest over a substantial period of time. In addition, the meaning of the changes seen in the physiological characteristics, in terms of fitness of the fish, should be determined. For example, are the characteristics related to reduced chances for survival, growth, or reproduction? This work points out the fact that concepts based on mammalian physiology should not be universally applied to fish without vertification.

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Evaluation of an Aerial Survey of Pacific Walruses (*Odobenus rosmarus divergens*)

JAMES A. ESTES[1] AND JAMES R. GILBERT[2]

National Fish and Wildlife Laboratory, Anchorage Field Station, Anchorage, Alaska 99503, USA
and
Center for Quantitative Science, University of Washington, Seattle, Wash. 98195, USA

ESTES, J. A., AND J. R. GILBERT. 1978. Evaluation of an aerial survey of Pacific walruses (*Odobenus rosmarus divergens*). J. Fish. Res. Board Can. 35: 1130–1140.

An aerial survey of Pacific walruses (*Odobenus rosmarus divergens*) was evaluated to determine the reliability of estimates of population abundance. The probability of detecting groups of walruses on the pack ice remained uniform to at least 0.93 km from the flight line, whereas the probability of detection decreased significantly beyond 0.23 km for walruses in the water. Walruses were more abundant along the ice-edge zone between 162 and 165°W than in other areas of the Chukchi Sea during September 1975. Few walruses were observed in consolidated pack ice north of the ice-edge zone or in ice-free water to the south. More walrus groups and larger mean group size were observed on September 8 than on other days. We estimated abundance for each day and all days combined using methods based on sample area and numbers of strip samples. Estimates varied among days by over an order of magnitude; this variation is attributed to the combined effect of chance sampling of an aggregated population and variation in the fraction of walruses hauled out. The coefficient of variation of the estimates ranged between 0.25 and 0.99. This imprecision was due to the aggregated distribution of walruses and the large variation in group size. Using the survey data as a basis for stratification, we calculated that, due to the high variability within strata, a sample size of 40% of the total area or 56% of the total available strips would be required to obtain 95% confidence limits within 10% of the estimate of total abundance. Variation contributed by observer error in estimating group size also is relatively unimportant to the precision of abundance estimates. Studies of natural history, particularly those oriented toward activity and habitat selection, would help investigators estimate bias due to the variable fraction hauled out and design surveys based on meaningful strata. Estimates of total abundance based on limited survey efforts will provide information of little reliability.

Key words: aerial survey, aggregation, Chukchi Sea, estimation, population, stratification, walrus

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Nous avons évalué un relevé aérien de morses du Pacifique (*Odobenus rosmarus divergens*) dans le but de déterminer la fiabilité des estimations d'abondance des populations. La probabilité de détecter des groupes de morses sur la banquise demeure uniforme jusqu'à au moins 0,93 km de la trajectoire de vol, alors que la probabilité de détection diminue de façon significative à plus de 0,23 km quand les morses sont dans l'eau. Les morses sont plus abondants le long de la banquise entre 162° et 165° de longitude ouest que dans toute autre région de la mer Chukchi en septembre 1975. Dans la glace de pack au nord de la marge de la banquise ou dans les eaux libres de glace vers le sud, nous avons observé peu de morses. On a vu le plus grand nombre de groupes de morses et des groupes comprenant en moyenne plus d'individus le 8 septembre qu'à tout autre jour. Utilisant des méthodes fondées sur l'aire d'échantillonnage et le nombre d'échantillons de bandes, nous avons estimé l'abondance à chaque jour et pour tous les jours combinés. Les estimations varient d'un jour à l'autre de plus d'un ordre de grandeur; on attribue cette variation à l'effet combiné de l'échantillonnage aléatoire d'une population rassemblée et de la variation du nombre de morses hors de l'eau. Le coefficient de variation des estimations va de 0,25 à 0,99. La distribution par groupe des morses et la grande variation du nombre d'individus dans les groupes sont la cause de cette imprécision. En stratifiant les données du relevé, nous calculons que, à cause de la grande variabilité intrastrate, il faudrait des échantillons de 40% de l'aire totale ou de 56% de toutes les bandes disponibles

[1]Present address: National Fish and Wildlife Laboratory, Center for Coastal Marine Studies, Applied Sciences Building, University of California, Santa Cruz, Calif. 95064, USA.

[2]Present address: School of Forest Resources, University of Maine, Orono, Maine 04473, USA.

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pour obtenir des limites de confiance à 95% en-dedans de 10% de l'estimation de l'abondance totale. La variation provenant de l'erreur de l'observateur dans l'estimation de l'abondance des groupes est, elle aussi, relativement peu importante et n'affecte pas la précision des estimations. Des études de l'histoire naturelle, surtout de l'activité et du choix de l'habitat des morses, aideraient les chercheurs à estimer le biais causé par la fraction variable d'animaux hors de l'eau et à concevoir des relevés fondés sur des strates significatifs. Les estimations d'abondance totale fondés sur des relevés limités fourniront une information peu fiable.

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THE status of marine mammal stocks have been assessed by using one or more of four general techniques: direct sightings, catch per unit of effort, mark–recapture analysis, and analysis of catch statistics. Because of the wide distribution and generally remote and inaccessible habitat of many species of marine mammals, aerial surveys have been used extensively. However, there is usually little information on the reliability of data from these surveys.

In this paper we evaluate several factors that contribute to the uncertainty of estimates of absolute abundance of marine mammals. Specifically, we consider data gathered during an aerial survey of the Pacific walrus (*Odobenus rosmarus divergens*). The major importance of this example is that it may serve as a paradigm for other marine mammals, for which the identified sources of bias and variation probably are applicable.

Distribution of the Pacific Walrus

The distribution of walruses coincides closely with the edge of the polar ice cap (Fay 1974; Burns 1970; Brooks 1954). Walruses migrate extensively in response to seasonal variation in the distribution of pack ice. During late winter the population is at the southern extreme of its range, generally occupying the area from south and southwest of St. Lawrence Island to Bristol Bay. Virtually the entire population is then associated with the pack ice, and most of the animals are east of the International Date Line (IDL). After passing northward through Bering Strait in late spring, many walruses apparently move northwestward and spend summer in waters west of the IDL. By late summer, when the pack ice has receded into the Arctic Ocean, walruses are found from about Point Barrow west to Wrangel Island and along the ice edge of the East Siberian Sea to near the mouth of the Kolyma River. During late summer and fall some animals forsake the pack ice for traditionally used hauling areas along the coast. Most of these currently in use are on the northern coast of the Chukchi Peninsula and Wrangel Island.

Materials and Methods

We flew aerial surveys over the eastern half of the Chukchi Sea on September 1, 5, 8, 9, and 12, 1975, covering a distance of 7743 km in 30.2 h.

We selected the northern and eastern boundaries of the study area (Fig. 1) on the basis of prior observations that Pacific walruses are distributed at that time principally along the ice-edge zone west of Point Barrow (Fay 1957; Estes and Gilbert unpublished data). On the basis of the most recent estimate of ice distribution, 69°30′N (the southern boundary) was about 185 km south of the ice edge, and we assumed that most walruses in the open water would not be farther south. The area west of the IDL was surveyed by biologists from the Soviet Union (comprehensive report in preparation).

We allocated 4 d of survey to the ice edge and 1 d to the expanse of open water between the ice edge and 69°30′N. Before each survey, we used a table of random numbers to select without replacement the longitude of north–south oriented strip samples from a set of 360 strip units 1.85 km apart, for the region between 156 and 174°W.

During the survey over open water, five north–south oriented strip samples were flown in a northerly direction from 69°30′N through the ice-edge zone to the southern boundary of the consolidated pack ice. From that point we flew diagonally southwest to the southern end of the next north–south strip, and thus obtained 10 strip samples of the distribution and abundance of walruses in the open-water segment of the survey area. We also located the exact position of the ice edge at each point where it was crossed by the flight line of our aircraft. This information, in conjunction with data provided by the U.S. Navy Fleet Weather Facility, was used to determine the northern and southern extremities of later survey strips that were flown over the ice-edge zone.

We randomly selected the location of strip samples over the ice-edge zone in a similar way, except that the northern and southern extremities of these strips were determined by points 74 km north and 18.5 km south of the most recently observed ice edge. When the distance between two adjacent strips flown on a given day was >18.5 km, a diagonal strip was flown between them. When the distance was <18.5 km, we eliminated the diagonal strip to avoid overlapping with north–south strips and flew to the nearest end of the next north–south strip. In general, we anticipated the location of the ice edge accurately enough, although on several occasions we changed the end points of our transects slightly to accommodate unexpected shifts.

At the eastern end of the survey area, the edge zone was well compacted; consequently north–south strips of about 92.5 km were sufficient to cover the area between the consolidated pack and the southern extremity of dispersed drifting ice. The edge zone at the western end of the survey area was more diffuse, and several of the north–south strips were more than 185 km long. The sample strips on each day were chosen randomly from the entire study area and were independent of the locations of samples on other days. We flew 9, 14, 7, and 13 strip samples on September

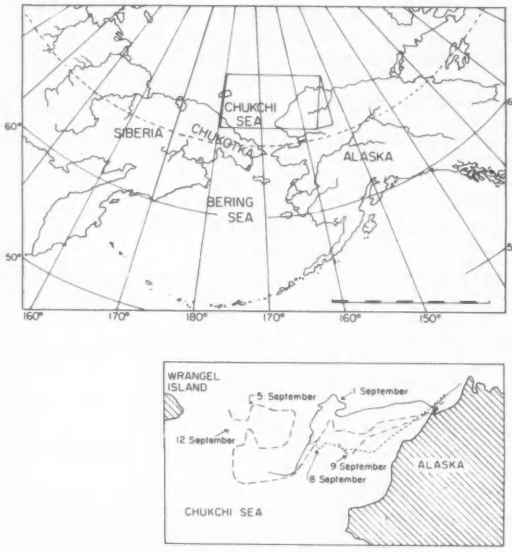

FIG. 1. Study area showing the southern extent of the ice-edge zone from September 1 to 12, 1975.

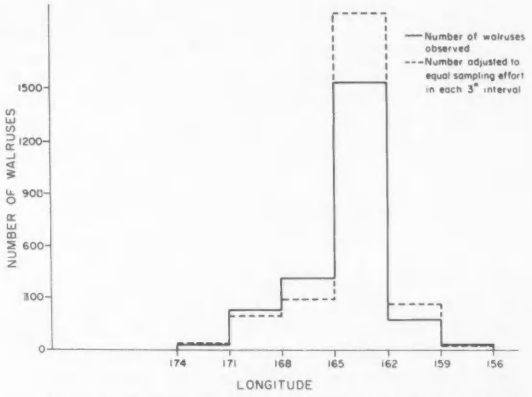

FIG. 2. Number of walruses observed (solid line) within longitudinal intervals of three degrees along the ice edge of the Chukchi Sea. Only data from September 5, 8, and 12, 1975 are included. Numbers were adjusted (broken line) by multiplying by $\bar{n}/n_i$, where n_i = the number of strip samples taken from the i^{th} interval of three degrees longitude and $\bar{n}$ is the mean of these values.

5, 8, 9, and 12, respectively. Of these, 5, 8, 4, and 8 were on random north–south lines.

We conducted the survey from a Lockheed P2V aircraft equipped with a Global Navigation System. This aircraft afforded excellent visibility from the nose bubble and offered reasonable safety. The aircraft was flown at an altitude of 150 m and at a speed of 275 km/h, except where visibility was inadequate because of a low cloud ceiling. When the ceiling descended below 60 m, the aircraft was flown above the fog and clouds on the predetermined flight line, until adequate viewing from 60 m or higher allowed resumption of the survey.

Two observers participated in the survey over open water, counting walruses from the nose bubble of the aircraft and recording the data. Four observers participated each day in surveys of the edge zone; two counted walruses, a third (seated behind the observers) recorded the data, and the fourth rested. To avoid fatigue, no person worked continuously at one task for more than 2 h and each person rested for at least 2 h during each flight.

The sample unit was a strip extending 0.93 km to each side of the aircraft. We counted each group of walruses observed within and outside the strip and counted or estimated the number in the group. To be included in a group, a walrus had to be within one body length of other walruses. The two observers divided the survey strip in half by an imaginary line directly beneath, and parallel to, the flight path of the aircraft. Animals observed within the 1.85-km-wide strip were classified further as occurring from 0.0 to 0.23, 0.23 to 0.46, and 0.46 to 0.93 km from the flight path. We determined these distances by sighting through the apex and the appropriate angle marked on protractors mounted at eye level on both sides of the nose bubble. The angle was adjusted for changes in altitude. The recorder kept a running log of time, location, and information provided by the observers. In addition, the recorder estimated ice cover in oktas (discrete categorizations of ice cover by increments of 1/8, 0/8 referring to ice free water and 8/8 to consolidated pack ice), the visibility, and the height of the cloud ceiling.

Results

DISTRIBUTION

The ice edge moved generally southward from September 1 when the survey was begun to September 12 when it was completed (Fig. 1). This movement was as great as 140–185 km in some areas. Furthermore, the ice edge was convoluted in the vicinity of the IDL, swinging north and crossing the Date Line at several locations.

Walruses were not distributed uniformly or randomly from east to west along the edge zone but were most abundant in the vicinity of 162–165°W (Fig. 2).

Large numbers of walruses were observed in all ice concentrations (Table 1). The data were pooled into four categories of ice cover (0/8, 1/8–3/8, 4/8–7/8, and 8/8) because estimates of ice cover were crude and were confounded by unknown differences among observers. This categorization was chosen because 0/8 and 8/8 represent end points of the continuum of ice cover and therefore can be exactly determined; the separation between 3/8 and 4/8 is somewhat arbitrary but was chosen because we believe that most observers can estimate with reasonable accuracy whether ice cover is >50% or <50%.

Mean group size was highest in 4–7 oktas ice concentration, but highest group sizes occurred in different ice concentration categories on different days (Table 1). Because no consistent pattern of distribution of group size in relation to ice concentration existed, future censuses could not be stratified over ice concentration.

TABLE 1. Walrus distribution and mean group size compared by ice cover. Only animals observed within the 1.86-km-wide strip during the ice-edge surveys are shown.

	Ice cover (oktas)									
	0/8		1/8–3/8		4/8–7/8		8/8		All ice classes	
Date (Sept. 1975)	No.	Mean group size	No.	Mean group size	No.	Mean group size	No.	Mean group size	No.	Mean group size
5	32	8.0	15	1.5	78	9.8	0	—	125	5.7
8	26	8.7	1166	17.9	1788	34.4	243	11.7	3214	23.0
9	30	15.0	30	5.0	138	8.1	2	1.0	200	7.4
12	0	—	55	5.5	275	30.6	0	—	330	17.4
Summary	88	9.8	1264	13.9	2279	26.5	234	10.6	3869	18.6

Of those walruses observed from the ice edge northward, 98.6% fell in the southernmost 6 deciles (Fig. 3), compared with an expected 60% if the distribution had been uniform. These data suggest that our sample area included all or most of the walruses located in the general vicinity of the ice edge, i.e. few animals were farther north in the consolidated pack. There was no comparable distributional pattern of animals in the open water south of the ice edge (Fig. 3), suggesting that we may have missed a considerable number by limiting the southward extent of the strips to 69°30′N. This suggestion is partly negated by the fact that few animals were seen during the open-water survey or during open-water portions of strips flown over the ice edge.

PROBABILITY OF DETECTION

To determine whether probability of detection was a function of right-angle distance from the flight line, the numbers of walruses observed in three substrips, 0–0.23, 0.23–0.46, and 0.46–0.93 km from the flight path were compared (Table 2). Data from both sides of the aircraft were pooled to increase sample size and to reduce any confounding effect of observer variation. The observed distribution of groups on ice was not significantly different from the expected distribution, indicating that the probability of detecting groups on ice was nearly constant to a distance of at least 0.93 km (as concluded also by Kenyon 1972). On the other hand, the distribution of groups in the water differed significantly from a uniform distribution, indicating that the probability of detecting groups in water decreased at distances <0.93 km. These results are supported by a comparison of sightings beyond 0.93 km over the water (3% of all groups seen) and on the ice (65% of all groups seen). The distribution of individuals on the ice and in the water departed further from uniform than did the distribution of groups. Since the distribution of groups on ice was not significantly different from uniform, the dramatic departure of the distribution of individuals from a uniform distribution cannot be the result of a nonuniform probability of detection. It must be due to either a random error effect of the highly aggregated distribution of individuals or the effect of positive bias in estimating group size as a function of distance. The latter explanation is rejected because separate examination of data from the right and left sides of the aircraft indicated different patterns in depar-

TABLE 2. Chi-square tests of goodness of fit with uniform distributions of walruses observed over distance from aircraft.

	Distance from flight track (km)			
	0–0.23	0.023–0.046	0.046–0.093	χ_2^2
		On ice		
Groups				
Observed	31	41	80	1.7
Expected	38	38	76	
Individuals				
Observed	442	944	2323	372.5[a]
Expected	927.25	927.25	1854.5	
		In water		
Groups				
Observed	26	17	13	18.9[a]
Expected	14	14	28	
Individuals				
Observed	66	53	39	42.6[a]
Expected	39.5	39.5	79	

[a] $P(\chi_2^2 > 9.2) = 0.01$.

TABLE 3. Observed and expected numbers of walruses over ice separated into right and left sides of the aircraft.

	Distance from flight track (km)			
	0–0.23	0.23–0.46	0.46–0.93	χ_2^2
Right side				
Observed	320	535	499	161.8[a]
Expected	338.5	338.5	677	
Left side				
Observed	122	409	1824	779.8[a]
Expected	588.75	588.75	1177.5	

[a] $P(\chi_2^2 > 9.2) = 0.01$.

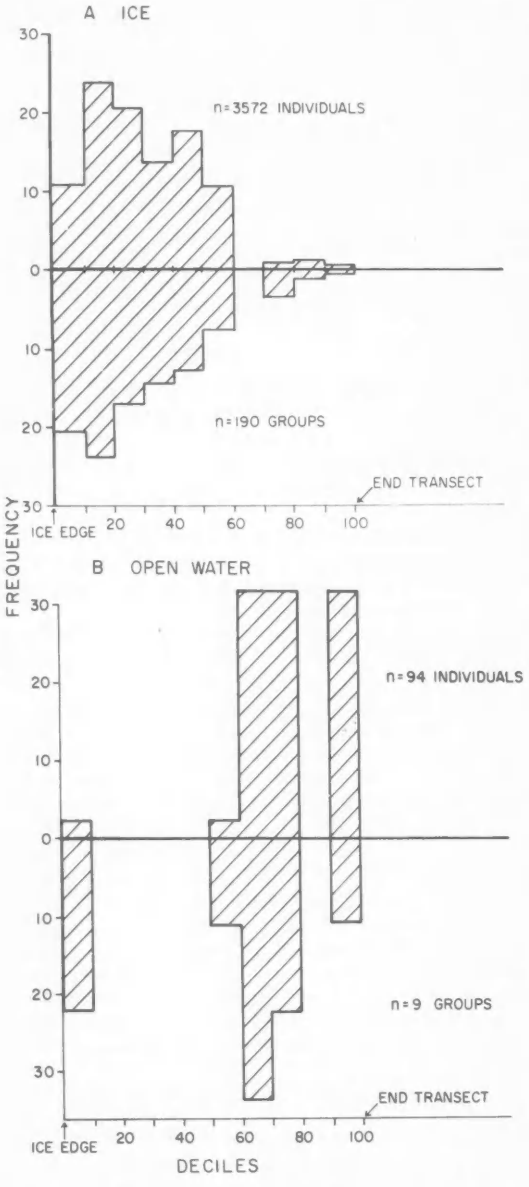

FIG. 3. Frequency distributions of the number of walruses and the number of groups of walruses observed in decile segments of the survey strips from the ice edge over the ice (A) and over water (B), near the ice edge of the Chukchi Sea September 5–12, 1975.

ture from the uniform distribution (Table 3), and neither is similar to the pattern indicated by the pooled data. The effect of grouping produces a similar, but less marked, departure from the uniform distribution of individuals in water. The fact that mean group size was much less in water than on ice (Table 4) accounts for this difference. Because the probability of detecting a group on ice was uniform up to 0.93 km from the flight line, the 1.86-km strip width was sufficiently narrow to presume each group within the strip had an essentially equal probability of detection. Therefore this strip width could be used in future censuses over pack ice. Conversely, because the probability of detecting a group in the water decreased beyond 0.23 km, future censuses of walruses should be confined to a strip width no greater than that distance to either side of the flight line over water.

TABLE 4. Numbers of walruses and mean group size, on ice and in water near the ice edge of the Chukchi Sea.

Date (Sept. 1975)	On ice		In water	
	Total no.	Mean no. per group	Total no.	Mean no. per group
1	—	—	42	2.0
5	106	10.0	19	1.6
8	3150	26.7	63	2.9
9	161	8.9	38	4.2
12	292	48.7	38	2.9

VARIATION AMONG DAYS

More walruses were hauled out on the ice on September 8 than on September 5, 9, or 12 (Table 5). Analysis of variance of the number of groups observed per transect indicated a significant difference among days ($F_{3,\ 39} = 4.429$; $P < 0.01$) and the total number of groups observed was approximately an order of magnitude greater on September 8 than on the other three days (Table 6). Furthermore, when the observed numbers of walruses in water and on ice each day were tested against the numbers expected if there were no differences among days, a highly significant difference was obtained ($\chi_3^2 = 208.56$; $P < 0.01$). The same analysis, exclusive of the data from September 8, indicated no significant difference among days ($\chi_2^2 = 5.91$; $P > 0.05$).

The frequency distribution of group size observed on September 8 compared with the combined days of September 5, 9, and 12 (Fig. 4) indicates that a greater proportion of large groups occurred on September 8. Group size on ice on September 8 was distributed approximately as a truncated negative binomial (Table 7). The walruses were aggregated on September 8 as indicated by the small value of the parameter k. On September 5, 9, and 12 only a single group larger than 50 walruses and only 6 groups larger than 25 walruses were observed, whereas on September 8, 12 groups larger than 50 and 62 groups larger than 25 were seen. The high mean group size on September 12 (Table 4) resulted from the sighting of a group of 225 individuals on that

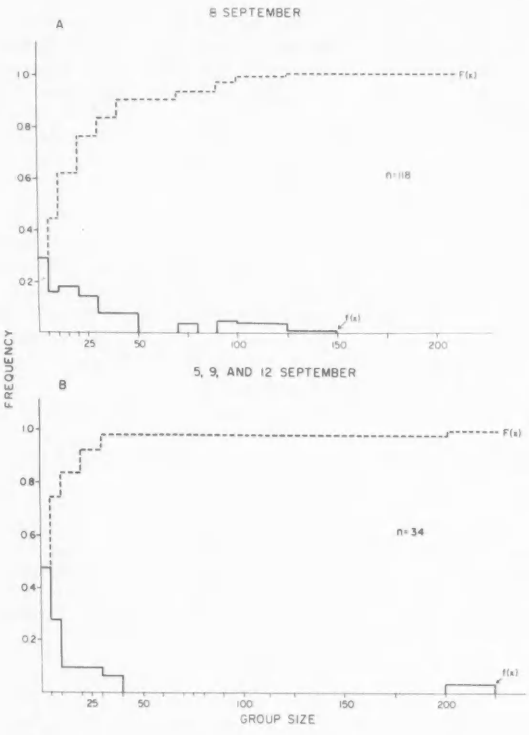

FIG. 4. Frequency distribution of walrus group size observed in the Chukchi Sea during September 1975. Broken line F(X) shows the cumulative frequency distribution.

day. Mean group size of animals in the water was similar on different days. However, the total number of walruses seen in the water was nearly twice as large on September 8 as it was on any other census over ice. This observation suggests that most of the walruses on September 5, 9, and 12 were not only in the water, but were also either beneath the surface or at some location where they were not observable (e.g. beneath the ice or outside the area sampled).

ESTIMATES OF ABUNDANCE

We estimated the total abundance of walruses separately for the ice-edge zone and the open-water area. Because strip samples flown each day in the ice-edge zone were independent, we could derive and compare three independent estimates of walrus abundance in that area.

We derived estimates of abundance using only the north–south random samples and using both these samples and those from diagonal flight lines. We employed two methods to estimate the abundance of walruses with each set of samples. In the first (Method I), we calculated a modified ratio of means estimator whereby the density of walruses ($\hat{R}$) is equal to $\Sigma y_i/\Sigma x_i$ with variance (D. S. Robson, Cornell University, and W. S. Overton, Oregon State University, personal communication)

$$(1) \qquad s_{\hat{R}}^2 = \{\Sigma(y_i^2/x_i) - \hat{R}\Sigma y_i\}/(n-1)(\Sigma x_i),$$

where y_i = the number of walruses in strip i, and x_i = the area of strip i. This method assumes that both the expected number of walruses and the variance of number of walruses are proportional to sample area. These relationships between number and area have been demonstrated in other censuses of large mammals (e.g. Siniff and Skoog 1964).

The abundance $\hat{T}_y = \hat{R}A$ with variance

$$(2) \qquad V(\hat{T}_y) = A(A - \Sigma x_i)s_{\hat{R}}^2,$$

where A = the area of the sample space.

In the second method (Method II) of estimating the total abundance of walruses, we calculated the mean number of walruses per strip sample ($\bar{y}$) and extrapolated this to a total population estimate by multiplying by the total number of possible strip samples (N). The variance was estimated as (Cochran 1963)

$$(3) \qquad V(\hat{T}_y) = N(N-n)s_y^2/n,$$

where s_y^2 = variance of y_i (number of walruses per strip), n = number of sample strips counted, and N = total number of 1.85-km-wide sample strips in the area.

Method II could be used because the abundance of walruses (y_i) was independent of sample area (x_i) (Table 8). This lack of correlation likely was the result of high residual variability due to (i) the concentration of walruses between 162 and 165°W (Fig. 2); (ii) stochastic variation caused by the highly contagious distribution of walruses (Table 7); and (iii) the concentration of walruses near the ice edge (Fig. 3), rather than dispersal throughout the pack ice. Therefore it was appropriate to treat each strip across the ice edge as a random sample of the ice front between 156 and 174°W. The length of each strip in this method was arbitrary, as long as it spanned the width of the ice-edge zone in which walruses were concentrated.

We modified both methods to estimate the number of walrus groups, then estimated walrus number ($\hat{T}_y$) from the product of number of groups ($\hat{T}_G$) and mean group size ($\bar{G}$). This estimate $\hat{T}_y = T_G\bar{G}$ has an estimated variance (Goodman 1960)

$$(4) \quad V(\hat{T}_y) = V(\bar{G}\hat{T}_G) = \bar{G}^2V(\hat{T}_G) + \hat{T}_G^2V(\bar{G}) - V(\hat{T}_G)V(\bar{G}),$$

where $V(\bar{G})$ = variance of mean group size. By increasing sample size, $V(\bar{G})$ could be reduced. We had hoped to accomplish this by combining observations from all four survey days of the ice edge. However, analysis of variance of group size among days indicated a significant difference ($F_{3,\ 203} = 3.81$; $P < 0.025$). Therefore only data on group size for a particular day is used to estimate total number of walruses.

We also made a separate set of estimates which were based only on the truly random N–S strip samples (elimi-

nating diagonal samples). While we believe the diagonal samples were at least representative, virtually nothing is known about the effect of such sampling on precision and bias (Eberhardt 1978).

Estimates of abundance (Table 9) indicate that most of the walruses were closely associated with the ice-edge zone. Population estimates ranged from 818 to 1760 in the open-water area, while in the ice-edge zone these estimates ranged from 2475 to 100 568. All estimates of total abundance lacked precision (Table 9). The most precise coefficient of variation $\{s(\hat{T})/\hat{T}\}$ was 0.25; most others ranged between 0.50 and 0.80. Method I was consistently more precise than Method II. Estimates derived from products of mean group size and number of groups were more precise only for September 8, when mean group size was much larger than on other days (Table 4).

In the ice-edge zone, there was a high variation among the three independent estimates (i.e. days) of abundance. On September 8, estimates were approximately an order of magnitude greater than those for any other day.

Discussion

Methods for estimating walrus abundance by direct observation from aircraft are limited to line transects and quadrats. Walrus groups frequently are clustered such that measurement of right-angle distance from the flight line to each group is impossible from a fast-moving aircraft. For this reason, and because of certain theoretical considerations (Eberhardt 1978), we rejected the various line transect estimators.

We therefore employed a strip sample, which is a

TABLE 5. Data summary including area and location of survey strips, and observations of walruses by individuals and group size on ice and in water.

Date (1975) and transect no.	Total area (km²)	Sample[a] area (km²)	Limits of strip				No. of groups		No. of individuals	
			Beginning		End					
			Lat.	Long.	Lat.	Long.	Ice	Water	Ice	Water
Sept. 1[b]										
1	192	127	70°16′	161°06′	71°13′	161°06′	—	—	—	—
2	319	302	71°13′	161°06′	69°49′	163°06′	—	1	—	3
3	340	316	68°38′	163°06′	71°17′	163°06′	—	20	—	39
4	412	316	71°17′	163°06′	69°30′	165°57′	—	—	—	—
5	429	343	69°30′	165°57′	71°35′	165°57′	—	—	—	—
6	449	346	71°35′	165°57′	69°30′	167°30′	—	—	—	—
7	405	333	69°30′	167°30′	71°28′	167°30′	—	—	—	—
8	432	340	71°28′	167°30′	69°30′	167°27′	—	—	—	—
9	154	86	69°30′	169°27′	70°15′	169°27′	—	—	—	—
10	168	0	70°15′	169°27′	69°49′	171°27′	—	—	—	—
Sept. 5[c]										
1	175	106	71°43′	159°09′	70°52′	159°09′	3	8	7	11
2	333	333	70°52′	159°09′	71°48′	163°15′	—	1	—	4
3	271	271	71°48′	163°15′	70°27′	163°15′	2	2	29	3
4	370	288	70°27′	163°15′	71°54′	166°27′	—	—	—	—
5	281	247	71°54′	166°27′	70°32′	166°27′	3	—	68	—
6	367	312	70°32′	166°27′	71°38′	170°45′	—	—	—	—
7	484	405	71°38′	170°45′	69°17′	170°45′	—	1	—	1
8	504	436	69°17′	170°45′	71°37′	173°03′	2	—	2	—
9	436	374	71°37′	173°03′	69°30′	173°03′	—	—	—	—
Sept. 8[c]										
1	175	175	71°19′	156°09′	72°10′	156°09′	1	—	2	—
2	316	316	72°10′	156°09′	70°53′	158°57′	1	1	3	2
3	171	137	70°53′	158°57′	71°43′	158°57′	—	2	—	3
4	285	285	71°43′	158°57′	70°34′	161°21′	22	9	491	18
5	175	175	70°34′	161°21′	71°25′	161°21′	9	5	160	21
6	340	298	71°25′	161°21′	70°11′	163°46′	19	2	282	2
7	189	189	70°07′	164°03′	71°02′	164°03′	35	—	1489	—
8	175	175	71°02′	164°03′	70°24′	165°45′	9	1	380	8
9	137	137	70°24′	165°45′	70°50′	165°45′	5	1	120	5
10	171	113	70°50′	166°00′	70°00′	166°00′	7	1	48	4
11	195	123	70°00′	166°00′	70°50′	167°18′	1	—	10	—
12	171	79	70°50′	167°18′	70°00′	167°18′	5	—	160	—
13	137	24	70°00′	167°18′	70°52′	168°14′	—	—	—	—
14	154	96	70°50′	168°18′	70°05′	168°18′	4	—	5	—

TABLE 5 (*Concluded*)

Date (1975) and transect no.	Total area (km²)	Sample[a] area (km²)	Limits of strip: Beginning Lat.	Beginning Long.	End Lat.	End Long.	No. of groups: Ice	No. of groups: Water	No. of individuals: Ice	No. of individuals: Water
Sept. 9[c]										
1	171	171	71°45′	158°15′	70°55′	158°15′	2	—	18	—
2	178	178	70°55′	158°15′	71°27′	160°18′	—	—	—	—
3	171	171	71°27′	160°18′	70°37′	160°18′	1	2	3	30
4	171	171	70°32′	160°42′	71°22′	160°42′	1	—	8	—
5	432	0	71°22′	160°42′	70°05′	165°42′	—	—	—	—
6	171	154	70°05′	165°42′	70°55′	165°42′	13	6	131	7
7	165	69	70°55′	165°42′	70°10′	166°47′	1	1	1	1
Sept. 12[c]										
1	549	226	69°30′	173°45′	72°10′	173°45′	1	—	25	—
2	535	535	72°10′	173°45′	70°05′	168°33′	2	1	40	2
3	220	220	70°06′	168°33′	71°05′	168°33′	1	2	225	4
4	171	93	71°05′	168°18′	70°15′	168°18′	—	—	—	—
5	182	127	70°15′	168°18′	71°03′	167°09′	—	—	—	—
6	171	93	71°05′	167°09′	70°15′	167°09′	—	—	—	—
7	195	96	70°15′	167°09′	70°50′	165°00′	—	—	—	—
8	171	79	70°50′	165°00′	70°00′	165°00′	—	—	—	—
9	171	93	70°00′	165°00′	70°46′	164°18′	—	—	—	—
10	182	99	70°46′	164°18′	69°56′	164°18′	—	7	—	13
11	504	333	69°56′	164°18′	71°43′	159°03′	2	2	2	2
12	171	171	71°43′	159°03′	70°53′	159°03′	—	—	—	—
13	171	171	70°53′	158°42′	71°43′	158°42′	—	1	—	17

[a]Area over the ice edge where visibility was ≥0.93 km.
[b]Open-water survey.
[c]Edge-zone survey.

modified quadrat. There are several advantages to quadrat samples, probably the most important of which is that if the quadrats are randomly selected, an unbiased estimate is free from any constraints regarding the distribution of animals in space. Furthermore, the method is relatively simple in that observers are not burdened with the task of making a complex series of observations on each animal or group of animals encountered. Seber (1973) pointed out that, other factors being equal, strips generally provide more variable estimates than do square quadrats. However, for the specific problem of aerial surveys of walruses, a strip consisting of a single flight line is the only practical quadrat shape because the survey aircraft is not capable of obtaining

TABLE 6. Variation among days in the number of groups of walruses observed.

Date (Sept. 1975)	Total no. of groups observed	Mean no. of groups observed per sample ($\bar{x}$)	s_x
5	10	1.11	1.36
8	118	8.42	10.41
9	18	2.57	4.65
12	6	0.46	0.77

TABLE 7 Comparison between the observed distribution of walrus group size on September 8, 1975 with that expected if the distribution were truncated negative binomial.

Group size	Observed frequency	Frequency expected with truncated negative binomial[a]
1	9	7.03
2	7	6.02
3	11	5.76
4	3	4.83
5	4	4.43
6–7	1	7.88
8–9	2	6.89
10–15	19	14.38
16–25	18	19.03
26–35	16	12.71
36–45	8	7.97
46–55	8	6.26
56–65	0	4.51
66–75	1	3.28
76–85	2	2.40
86–95	0	4.62
96–112	5	
113–137	3	
138+	1	
Total	118	118

[a]$\bar{w} = 0.0262$; $k = 0.6422$; $\chi_{15}^2 = 27.20$ $(0.05 > P > 0.01)$.

TABLE 8. Coefficients of linear correlation between strip length (sample area) and the number of walruses observed.

Date (Sept. 1975)	Correlation coefficient (r)
5	−0.352
8	0.007
9	0.210
12	0.254

mutually exclusive and exhaustive coverage of any quadrat design other than a single flight line. Additionally, even if such coverage were possible, the intense noise produced by the P2V aircraft would disrupt animals in contiguous strips before they could be surveyed.

STATISTICAL PROBLEMS

Our estimates of total abundance of walruses are disappointingly imprecise (Table 9). This lack of precision is due only in part to the relatively small area or number

TABLE 9. Estimates of the total abundance of walruses in open water (48 842 km², 220 possible samples) and in the ice-edge zone (54 896 km², 360 possible samples).

Region and type of estimate	Based on estimates of individuals: Total abundance ($\hat{T}$)	Based on estimates of individuals: $S(\hat{T})$	Based on estimates combining groups and mean group size: Total abundance ($\hat{T}$)	Based on estimates combining groups and mean group size: $S(\hat{T})$
Open-water area				
September 1				
Method I[a]				
All samples (2509 km²)	818	684	818	665
N–S samples (1205 km²)	1 581	1 309	1 621	1 348
Method II[b]				
All samples (n = 9)	1 026	927	1 026	954
N–S samples (n = 5)	1 716	1 696	1 760	1 740
Ice-edge zone				
September 5				
Method I				
All samples (2772 km²)	2 475	1 606	2 475	2 170
N–S samples (1403 km²)	4 656	2 846	4 224	4 094
Method II				
All samples (n = 9)	5 000	2 737	5 000	2 871
N–S samples (n = 5)	8 568	4 480	7 773	4 650
September 8				
Method I				
All samples (2322 km²)	75 959	30 921	75 959	19 121
N–S samples (1101 km²)	100 568	57 689	85 848	29 222
Method II				
All samples (n = 14)	82 620	37 428	82 620	26 020
N–S samples (n = 8)	90 765	63 561	77 480	33 007
September 12				
Method I				
All samples (2336 km²)	7 755	4 551	7 755	5 920
N–S samples (1152 km²)	13 533	7 990	11 587	9 259
Method II				
All samples (n = 13)	9 138	6 130	9 138	6 743
N–S samples (n = 8)	12 780	9 915	10 942	8 402
September 5, 8, and 12				
Method I				
All samples (7430 km²)	27 000	10 731	27 000	7 097
N–S samples (3656 km²)	36 337	20 616	32 871	11 511
Method II				
All samples (n = 36)	36 680	15 127	36 680	10 191
N–S samples (n = 21)	41 486	24 551	37 528	12 937

[a]Estimates are based on mean density per square kilometre extrapolated to total area.
[b]Estimates are based on mean number per sample and total number of possible samples.

of strips sampled. Large-scale variation in walrus density across the study area (Fig. 2) is a contributing factor. In addition walruses are distributed in highly aggregated groups when hauled out on the pack ice (Tables 5, 6, 7; Fig. 4) and therefore samples of walrus density inherently are highly variable (Pielou 1969).

The size of all but the smallest groups was estimated rather than measured exactly. We categorized these estimates into multiples of 5, 10, or 25, depending on the estimated size of the group. This in effect added a second random variable a_i to each random variable b_i, where b_i is the exact number of walruses in the ith group and $a_i + b_i = c_i$, the categorized estimate of group size. If our estimates were unbiased, then $E(a_i) = 0$ and $E(c_i) = E(b_i)$ with variance

$$V(c_i) = \sigma_a^2 + \sigma_b^2, \tag{5}$$

if a_i and b_i are uncorrelated, or

$$V(c_i) = \sigma_a^2 + \sigma_b^2 + \rho\sigma_a\sigma_b, \tag{6}$$

if a_i and b_i have a correlation coefficient $\rho \neq 0$. Therefore, if c_i is an unbiased estimate of b_i, the effect of arbitrarily categorizing that estimate does not affect the accuracy of the estimate, but it does inflate its variance. The question then becomes, What is the magnitude of this increase in variance? Assuming c_i is unbiased and $\rho = 0$,

$$V(c_i) = V(b_i) + E(a_i^2). \tag{7}$$

Equation (7), in conjunction with the frequency distribution of group size, can be used to estimate the magnitude of the increase in variance of c_i contributed by a_i. We assume that all groups of size 1 through 10 were accurately counted, and that groups from 11 to 50 were categorized by multiples of 5, groups from 51 to 100 by multiples of 10, and groups >100 by multiples of 25. This is probably approximately correct. If it is also assumed that a_i is a bounded random variable, then from the frequency distribution of estimates of group size on September 8, $E(a_i^2) = 9.88$ and $V(c_i) = 1666$. Furthermore, if one relaxes the assumption that $\rho = 0$ to $\rho = 1$ (i.e. a_i and b_i are exactly correlated), $E(\rho\sigma_a\sigma_b) = 123$ when σ_b is estimated by the positive root of equation (6). Therefore, provided c_i is not grossly biased, categorization contributes <8% of the total variance in estimates of group size. The conclusion from this analysis is that the inability of observers to count each group precisely is of little consequence. This conclusion further implies that the imprecision of our estimates of total abundance is related almost entirely to distributional properties and behavioral characteristics of walruses rather than to problems associated with visual observations.

The striking differences among estimates for each day chiefly are due to variation among days in the fraction of walruses hauled out and to sampling by chance particular areas of the ice-edge zone where walrus abundance varied. There is little information from which to predict the fraction of walruses hauled out. For other pinnipeds, e.g. ringed seals (*Phoca hispida*) (Burns and Harbo 1972), Antarctic seals (Siniff et al. 1971; Gilbert and Erickson 1977), and harp seals (*Pagophilis groenlandicus*) (Sergeant 1971), the number basking on the ice has been correlated with weather conditions and time of day, and recent studies of walruses on land have shown a distinct cyclicity in numbers over about 10-d intervals (J. Taggart and C. Zabel, personal communication).

Therefore we cannot derive a single estimate of total abundance because of the unknown extent to which daily estimates were influenced by the fraction hauled out as opposed to chance sampling. If variation in the fraction hauled out was the major cause of differences among days, then the data from September 8 alone would provide the best (most precise and least biased) estimate. However, if chance location of the samples was the major cause of differences among days the best estimate (least biased) should be based on data combined from all three days. This dilemma cannot be resolved without the ability to predict temporal patterns of hauling out activity.

The effects of geographical variation in many instances can be reduced by stratification (e.g. Siniff and Skoog 1964). In retrospect, from the data in Table 5 (see also Fig. 2), it appears that walruses were most abundant between 161 and 168°W. Mean density of walruses in that area (Area I) taken over all days was 1.43 per km^2 (212 per strip sample) compared with 0.14 per km^2 (27 per strip sample) in the remaining area (Area 2). These data could form the future basis for stratified sampling if walruses concentrate in these areas each September.

Sampling effort could be allocated on the basis of area (using variance estimates from Method I) or number of samples (Method II). Optimum allocation of effort based on stratum area (A_h) and stratum variance (S_h^2) using Method I would be (Cochran 1963)

$$a_h = a(A_hS_h/\Sigma A_hS_h),$$

where A_h = area of stratum h, a_h = sample area allocated to stratum h, and a = total sampling area. Based on data in Table 5, with $A_1 = 13\,724$ km^2 and $A_2 = 41\,172$ km^2, optimum allocation is achieved when $a_1/a = 0.70$ and $a_2/a = 0.30$.

Optimum allocation based on the number of possible samples in each stratum (N_h) and stratum variance (S_h^2), using Method II, would be (Cochran 1963)

$$n_h = n(N_hS_h/\Sigma N_hS_h),$$

where N_h = number of strips in stratum h, n_h = sample number allocated to stratum h, and n = total number of strip samples. Since $N_1 = 90$ and $N_2 = 270$, optimum allocation would be achieved when $n_1/n = 0.69$ and $n_2/n = 0.31$.

This information can be used to determine the total sampling effort required to reduce the confidence interval around the estimate to a given value, using either Method I or II. For convenience we use a 95% confidence interval of $\pm 0.1\hat{T}$, where $\hat{T}$ = the estimate of total abundance with variance either (Method I)

$$V(\hat{T}) = \Sigma A_h(A_h - a_h)S_h^2/a_h$$

or (Method II)

$$V(\hat{T}) = \Sigma N_h(N_h - n_h)S_h^2/n_h$$

With Method I, we would require a sample area of 22 029 km^2 (40% of the total), including stratum 1 in its entirety, to obtain the desired confidence interval. Similarly, with Method II we would require 202 sample strips (56% of the total), again including stratum 1 in its entirety, to obtain the desired confidence interval.

It follows that even if the fraction of walruses hauled out is accurately known, the aggregated distribution of individuals into large groups of varying size inherently causes high variation among sample units despite the effectiveness with which stratification may be able to account for larger scale variation in density. Therefore, a large sample size will be required to precisely estimate abundance from any future walrus survey. Using data from Kenyon (1972) for a walrus survey in the Bering Sea, we reached a similar conclusion (J. A. Estes, unpublished data).

Acknowledgments

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Incidence, Microscopy, and Etiology of Exoskeleton Lesions in the Tanner Crab, *Chionoecetes tanneri*[1]

JOHN A. BAROSS, PATRICIA A. TESTER, AND RICHARD Y. MORITA

School of Oceanography and Department of Microbiology, Oregon State University, Corvallis, Oreg. 97330, USA

BAROSS, J. A., P. A. TESTER, AND R. Y. MORITA. 1978. Incidence, microscopy, and etiology of exoskeleton lesions in the tanner crab, *Chionoecetes tanneri*. J. Fish. Res. Board Can. 35: 1141–1149.

As part of a seasonal population survey of the tanner crab, *Chionoecetes tanneri* Rathbun (Brachyura:Majidae), off the Oregon coast over 1400 adult and juvenile crabs were examined. A significantly high incidence of exoskeleton lesions was observed among adult females (76% infected) and to lesser extent among adult males (29%). No lesions were observed on juvenile specimens. Multiple lesions, some of which extended through the exoskeleton, were frequently observed on the carapace and appendages. Scanning electron miscroscopy of normal and diseased exoskeleton showed dense populations of bacteria associated with lesions and with the thin epicuticle of undiseased crabs. This suggests microbial degradation and/or mechanical damage to the epicuticle as a prerequisite to exoskeleton lesion formation. Chitin-digesting bacteria were isolated from all lesions examined. Sixty isolates were examined as to their phenotypic similarities to known chitin-digesting species that have been implicated as causing exoskeleton lesions in various crustaceans. Twenty-eight of the isolates were luminous and capable of digesting chitin rapidly at low temperatures. These isolates were compared with known luminous bacteria and found to be a *Photobacterium* sp. that did not precisely compare with known species. The possibility that multiple, luminous exoskeleton lesions might have a significant effect on some aspect of *C. tanneri* behavior is considered.

Key words: exoskeleton lesions, crab, chitin, microbiology, crustacean, *Photobacterium*

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Un relevé saisonnier des populations de *Chionoecetes tanneri* Rathbun (Brachyura: Majidae) au large de la côte de l'Orégon comportait entre autres l'examen de 1 400 jeunes crabes et adultes. Nous avons observé une forte incidence de lésions sur l'exosquelette des femelles adultes (76%) et à un moindre degré chez les mâles adultes (29%). Les jeunes ne montrent pas de lésions. On observe souvent des lésions multiples, dont quelques-unes traversent l'exosquelette, à la fois sur la carapace et sur les appendices. La microscopie électronique à balayage révèle, sur des exosquelettes normaux et malades, de denses populations de bactéries associées aux lésions et à la mince épicuticule de crabes sains. Cela suggère une dégradation microbienne et/ou une lésion mécanique de l'épicuticule comme condition préalable à cette maladie. Nous avons isolé de toutes les lésions examinées des bactéries capables de digérer la chitine. Nous avons examiné 60 isolats en vue de détecter des ressemblances avec des espèces que l'on sait capables de digérer la chitine et qui causent des lésions à l'exosquelette de divers crustacés. Vingt-huit des isolats sont lumineux et capables de digérer la chitine rapidement à de basses températures. Nous avons comparé ces isolats avec des bactéries lumineuses connues et avons constaté qu'il s'agissait d'un *Photobacterium* sp. qui ne se compare pas exactement avec des espèces connues. Nous examinons la possibilité que des lésions lumineuses multiples de l'exosquelette puissent avoir un effet significatif sur certains aspects du comportement de *C. tanneri*.

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EXOSKELETON lesions have frequently been observed on many different marine crustaceans, particularly on commercially important neretic species such as the American lobster, *Homarus americanus* (Hess 1937; Rosen 1970; Young and Pearce 1975), the blue crab, *Callinectes sapidus* (Rosen 1967; Krantz et al. 1969; Cook and Lofton 1973), the king crab, *Paralithodes camtschatica* (Bright et al. 1960), and various penaeid shrimps, *Penaeus* spp. (Cook and Lofton 1973). The disease is characterized by progressive softening and pitting of the chitinous exoskeleton usually accompanied by blackening of the necrotic region. To what extent exoskeleton lesions are involved in mortalities of a natural population of crustaceans is not known. It is assumed, however, that since the disease is not transferred to the new exoskeleton during ecdysis, exoskeleton lesions are probably not an important cause of mortalities (Bright et al. 1960). However, if the lesions extend through all layers of the exoskeleton, there seems little doubt that pathogenic microorganisms could invade the hemolymph. There is substantial evidence that exoskeleton lesions and mortalities due to bacteremia are frequently caused by the same species of bacteria (Krantz et al. 1969; Lightner and Lewis 1975; Vanderzant and Nickelson 1973; Brinkley et al. 1976). *Aerococcus viridans*, a nonchitin-digesting bacteria that causes severe mortalities in lobsters and a mild disease syndrome in crabs, can enter these animals through ruptures in the integument (Stewart et al. 1969). Under the crowded conditions imposed on aquaria-held animals, the incidence of mortalities following shell lesions may exceed 70% of the population (Taylor 1948).

It is generally believed that chitin-digesting bacteria are the principal causative organisms of shell disease, and chitin-digesting *Vibrio* spp. (frequently called *Beneckea* spp.) have been isolated from all marine crustacean exoskeleton lesions properly examined (Hess 1937; Rosen 1967, 1970; Cook and Lofton 1973; Young and Pearce 1975). It is not precisely known what sequence of events leads to shell erosion; however, many investigators report that mechanical damage to the shell is the chief prerequisite to lesion formation (Rosen 1970). This is undoubtedly an oversimplification since the highest incidence of necrotic lesions have been observed in animals obtained from heavily polluted areas and from warm inshore waters during the summer (Krantz et al. 1969; Pearce 1972; Young and Pearce 1975).

During the course of a population survey of the deep-water crab *Chionoecetes tanneri* (Brachura:Majidae) off the coast of Oregon, it was noted that a high percentage of adult specimens showed multiple and frequently quite severe exoskeleton lesions. This paper reports the incidence of shell disease among adult tanner crabs sampled over a 2-yr period, and the results of electron microscopic and microbiological analysis of exoskeleton lesions. Since this is the first reported instance of severe exoskeleton infection involving a natural population of a deep-water crustacean, it was of particular importance to compare the etiological agent of tanner crab lesions with the causative bacterial species of exoskeleton lesions in neretic species of crustaceans.

Materials and Methods

Chionoecetes tanneri were collected by otter trawl using the Oregon State University R.V. *Cayuse*. Samples were obtained from along the continental shelf and slope off the Oregon coast during eleven cruises from April 1973 to June 1975. Adult crabs were maintained on ice until they could be analyzed in the laboratory.

Exoskeleton segments from diseased and nondiseased male and female adult tanner crabs were excised using sterile techniques. Approximately 1-cm^2 sections of exoskeleton were either placed into Lib-X broth (Baross et al. 1974) or streaked onto Lib-X agar plates (1.2% Bacto-agar) supplemented with 1% purified chitin. The purified chitin was prepared from the pens of the squid (*Loligo opalescens*) using the procedure of Skerman (1959). All samples were incubated at 2°C for 14 d. Similar procedures were used to isolate chitinoclastic bacteria from exoskeleton lesions of dungeness crab (*Cancer magister*) obtained from Yaquina Bay, Oreg.; however, all bacteriological samples from *C. magister* were incubated at 15°C.

Chitinoclastic bacteria isolated from exoskeleton lesions were compared taxonomically with known strains of marine chitinoclasts that have been implicated as causative agents of exoskeleton lesions in a variety of marine crustaceans. The source and phenetic description of the chitinoclastic strains of *V. parahaemolyticus*, *V. alginolyticus*, and *V. anguillarum* used in this study have been previously described (Baross 1972). Strains of *Photobacterium* spp. were obtained from K. Nealson (Scripps Institution of Oceanography, University of California, LaJolla, Calif.), J. Makemson (San Francisco State University, San Francisco, Calif.), and the Oregon State University culture collection. The culture media and test reagents used to characterize marine chitinoclastic *Vibrio* species have been previously described (Morris et al. 1976) except that 3.5% Rila marine salts (Rila Products, Teaneck, N.J.) was used in place of either 3% NaCl or natural seawater. In addition, lipase was tested using the Tween-80 medium of Sierra (1957) supplemented with 3.5% Rila salts. Culture morphology and motility were observed by phase-contrast microscopy, using wet-mount preparations. Flagella stains were prepared with the Leifson stain (Difco) according to the procedures of Leifson (1951). Cells were fixed in 2% formalin prior to staining.

The taxonomic schemes of Reichelt and Baumann (1973) and Reichelt et al. (1977) were used to taxonomically identify luminous organisms isolated from tanner crab lesions. The substrates acetate, D-alanine, cellobiose, glucose, D-gluconate, D-glucuronate, DL-lactate, maltose, mannitol, L-proline, propionate, pyruvate and D-xylose were added as the sole source of carbon to a basal medium containing 3.5% Rila salts, 0.1% NH_4Cl, 0.005% $FeSO_4 \cdot 7H_2O$, 1.5% Bacto-purified agar (Difco), and the pH was adjusted to 7.5 using 0.5% Tris-HCl (Sigma). All substrates were sterilized separately and added to the basal medium at a final concentration of 0.2%. All media were incubated at 5°C for up to 10 d.

Excised 1-cm^2 samples were fixed for 8 h at 5°C in cold 3.5% Rila salts containing 3% gluteraldehyde (Sigma). The fixed samples were thoroughly washed in distilled water and quick frozen with dry ice and butanol. These frozen

TABLE 1. Incidence of exoskeleton lesions in tanner crabs (*Chionoecetes tanneri*) sampled off the Oregon coast from April 1973 to June 1975. *N* = number sampled, *I* = number infected.

	Adult females		Adult males		Juveniles	
Year	N	I	N	I	N	I
1973	135	99	78	15	557	0
1974	147	120	44	22	341	0
1975	8	2	2	1	102	0
Total observed	290	221	124	38	1000	0
% infected		76%		29%		0%

sections were then freeze-dried with a Virtis lyophilizer and mounted on scanning electron microscope stubs with silver paint (Testers) as an adhesive. The stubs were coated under vacuum with a 10–20-nm-thick layer of gold–palladium metal. The samples were viewed with an International Scientific Instruments Mini-SEM, Model MSM-2 scanning electron microscope.

Results

From April 1973 to June 1975 adult and juvenile tanner crabs were obtained from nine cruises off the coast of Oregon. A very high incidence of exoskeleton lesions was observed in adult specimens, particularly among females (Table 1). However, these data do not really indicate the magnitude of infection. In general, adult females showed multiple lesions, many of which overlapped so that enumeration was impossible. Frequently, the entire dorsal surface of the carapace was black and markedly softened and pitted from severe infection. In contrast, most of the infected males did not have multiple lesions and the lesions were clearly not as developed or as extensive as found on the females. Moreover, in contrast to the females, which generally showed multiple lesions on the carapace and the appendages, the lesions on male crabs were frequently located on the chelipeds and walking appendages, but rarely on the carapace.

A typical female tanner crab with multiple and expansive lesions is shown in Fig. 1. These lesions are typical of exoskeleton lesions found on other crustaceans and show irregular areas of black necrosis and destruction of the exoskeleton with a concomitant black line surrounding the necrotic area (Fig. 2). Also, it was not unusual to find lesions on female crabs that extended through all of the layers of the exoskeleton. In some instances a significant portion (over 2 cm) of an appendage was completely eroded (Fig. 3), thus allowing an opening for possible infection and fatal bacteremia, an observation, however, that was not unequivocally made.

Diseased and undiseased sections of tanner crab exoskeleton were examined by scanning electron microscopy. The thin epicuticle layer that covers normal, undiseased areas of the exoskeleton is shown in Fig. 4. The thin epicuticle layer can be seen to harbor high numbers of bacteria, detrital material, and possibly diatoms. The breaks and partial separations of the epicuticle are due to the fixation and preparation of exoskeleton samples for electron microscopy. Some preliminary experiments were conducted to determine the numbers of viable bacteria associated with the epicuticle. Portions of undiseased exoskeleton with known dimensions were vigorously shaken with sterile sand and Rila salt diluents to dislodge attached microorganisms. Bacterial counts between 10^3 and 10^6 per cm^2 were consistently obtained. The importance of this information became apparent upon closer examination of the exoskeleton layer under the epicuticle. This was accomplished by picking away sections of epicuticle with a needle. Figure 5 shows a typical nondiseased exoskeleton surface. Note that the

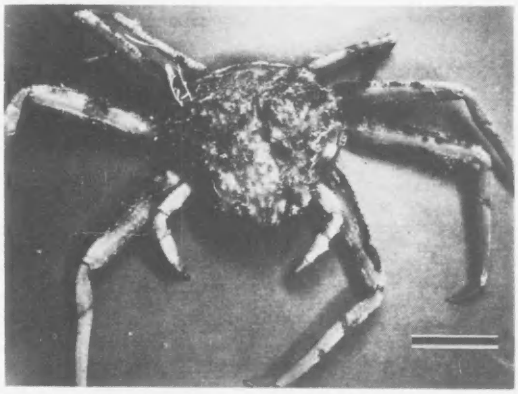

FIG. 1. Adult female tanner crab (*Chionoecetes tanneri*) showing multiple and expansive exoskeleton lesions on the carapace and appendages. Bar = 50 mm.

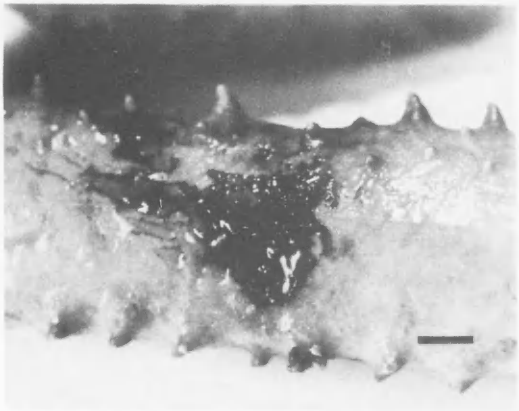

FIG. 2. Necrotic lesion on the cheliped of an adult tanner crab (*C. tanneri*) showing erosion and blackening within and surrounding the area of necrosis. Bar = 5 mm.

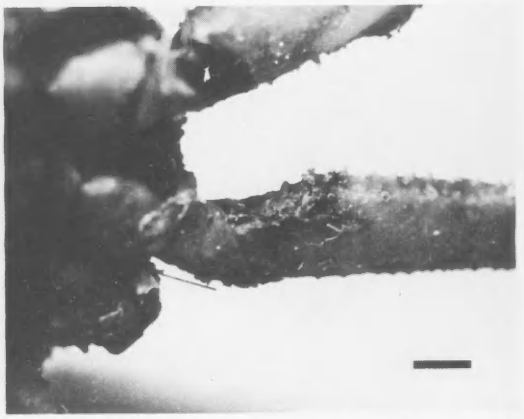

FIG. 3. Severe erosion on a segment of a walking appendage of an adult tanner crab (*C. tanneri*) in which the necrosis extended through all layers of the exoskeleton. Bar = 10 mm.

FIG. 5. High-magnification scanning electron micrograph of a nondiseased portion of the exoskeleton that lies beneath the epicuticle of an adult tanner crab (*Chionoecetes tanneri*). Bar = 5 μm.

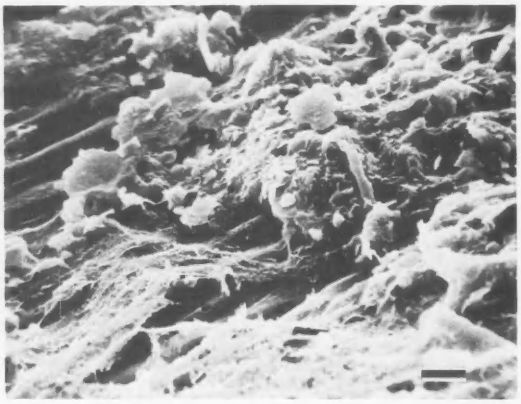

FIG. 4. High-magnification scanning electron micrograph of the epicuticle layer of a nondiseased portion of the tanner crab (*C. tanneri*) exoskeleton showing the dense populations of epiphytic detritus and microorganisms. Bar = 5 μm.

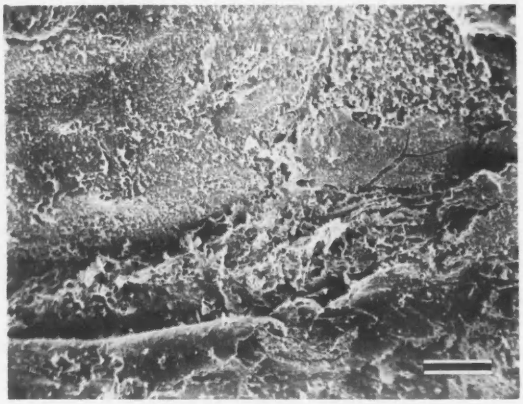

FIG. 6. Low-magnification scanning electron micrograph of an exoskeleton lesion on the appendage of an adult tanner crab (*C. tanneri*). Bar = 150 μm.

ridges are approximately 2–5 μm wide and if bacteria were colonized on this surface they would be quite visible. Bacteria were never noted beneath the epicuticle on undiseased shell, thus strongly indicating the possibility that the epicuticle serves as a protective shield against exoskeleton colonization and degradation by chitin-digesting bacteria.

Necrotic areas of the exoskeleton were typically free of any epicuticle layer or smooth regularly shaped ridges. This can be clearly seen in Fig. 6, which shows at low magnification a necrotic lesion that extends through all layers of the exoskeleton. Note particularly that the erosion progresses from layer to layer. Increased magnification of this necrotic lesion shows a very dense population of bacteria with very obvious bacterial colonization (Fig. 7). These levels of bacteria were observed on all lesions examined and appeared to form a confluent layer over the surface within the necrotic area.

All lesions sampled for bacteria yielded chitinoclasts. In general, based on colony morphology, there appeared to be one species of chitin digester associated with a lesion. In addition, a variety of psychrotrophic nonchitin digesters were isolated from each lesion sampled. Many of these isolates were gram positive and resembled members of the *Arthrobacter–Corynebacterium* group and various genera within the *Micrococcaceae*, including *Planococcus* and *Aerococcus* sp. These same organisms were found to be the dominant types isolated from the epicuticle of nondiseased crabs.

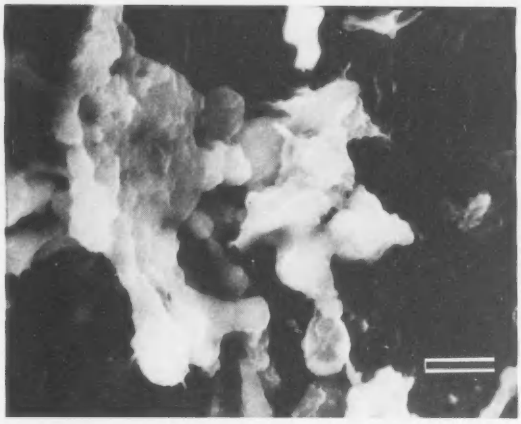

FIG. 7. High-magnification scanning electron micrograph of a tanner crab (*Chionoecetes tanneri*) exoskeleton lesion showing dense populations of colonized bacteria. Bar = 2 μm.

Representative chitin-digesting bacteria, isolated from necrotic lesions of 35 different crab specimens, were compared with known chitin-digesting *Vibrio* species and chitinoclasts isolated from necrotic lesions of dungeness crabs (Table 2). In general, the tanner crab chitinoclasts were phenotypically heterogeneous and there appeared to be at least three distinct groups. However, the dominant group (28 isolates) was found to be luminescent and capable of hydrolyzing chitin at low temperatures (below 10°C) much more rapidly than the other chitinoclasts. A small group of slow chitin digesters (3 wk at 10°C) consisting of eight isolates were facultative halophiles and could grow in 0% as well as 10% NaCl and resembled *Moraxella* species. The remaining isolates were psychrotrophic vibrios that phenotypically resembled *V. anguillarum* except they could grow well at 0°C; similar *Vibrio* spp. were also isolated from the epicuticle of nondiseased crabs. All of the isolates from dungeness crab lesions were phenotypically identical to *V. anguillarum*.

The high incidence of luminous isolates that were very rapid chitin digesters strongly suggested that these organisms might play a prominent role in the formation of exoskeleton lesions on *Chionoecetes tanneri*, particularly if these organisms were found to be a single species. Thus, these isolates were further characterized and phenotypically compared with the recognized species of luminous bacteria by using the determinative scheme of Reichelt and Baumann (1973) and Reichelt et al. (1977). These data are summarized in Table 3. The tanner crab luminous isolates clearly represent a single species of *Photobacterium* that is not identical to any of the known *Photobacterium* species. These isolates appear to be quite similar but not identical to *P. phosphorium* and *P. fisheri*.

Discussion

All of the previous reports on exoskeleton lesions of marine crustaceans involved species that reside in shallow waters generally not exceeding 200 m. In contrast, *C. tanneri* is a deep-water species that inhabits the benthic environment at depths between 500 and 2000 m off the Oregon and Washington coasts. This species is not known to migrate inshore at any phase of its life cycle. At these depths, the low in situ water temperature (2–3°C) and moderate hydrostatic pressures (2.02×10^7 Pa) are quite restrictive to the growth of the most commonly isolated chitinoclastic bacteria from inshore environments including crustacean exoskeletons. It could be assumed, therefore, that the causative organisms of *C. tanneri* lesions are physiologically different from their counterparts on neretic species of crustaceans. Indeed, this was found to be true and the major chitin-digesting bacteria isolated from tanner crab lesions were not significantly related to the *Vibrio* spp. most frequently isolated by other investigators.

Vibrio parahaemolyticus and *V. alginolyticus*, which have been inculpated as causing exoskeleton lesions and occasionally a fatal bacteremia to various species of crustaceans (Krantz et al. 1969; Vanderzant and Nickelson 1973; Brinkley et al. 1976), are mesophilic and quite barosensitive (Baross et al. 1975; Schwarz and Colwell 1974) and thus would be incapable of growth under the in situ conditions imposed on *C. tanneri*. *Vibrio anguillarum*, a known fish, molluscan, and crustacean pathogen, is probably the most commonly isolated chitinoclast from inshore faunal, water, and sediment samples. In the present study, *V. anguillarum* was evidently the only chitinoclastic species isolated from dungeness crab (*Cancer magister*). Occasionally we have found lesions sampled from various penaeid shrimp and the common rock crab, *C. productus*, to be caused by *V. anguillarum*. Moreover, it is presumed that if appropriate taxonomic tests were employed, *V. anguillarum* and related species would probably be the most commonly isolated chitinoclasts from all exoskeleton lesions involving inshore species of crustaceans.

The term mesophile *sensu strictus* includes bacterial species that grow within the temperature range of approximately 10–45°C such as *V. parahaemolyticus* and *V. alginolyticus*. *Vibrio anguillarum* would be excluded from this group because of its intermediate temperature range of growth, which is from <5°C to approximately 35°C. This species is quite well adapted thermally to inshore environments but would either not grow or grow very slowly under the in situ conditions in the deep benthos. Indeed, the most commonly isolated organism from *Chionoecetes tanneri* that was capable of optimal growth and rapid chitin hydrolysis at in situ temperatures was a *Photobacterium* sp. Vibrios were uncommonly isolated from the tanner crab and it is presumed that these fermentative organisms play a minor role in the formation and progression of exoskeleton lesions in deep waters.

TABLE 2. Comparison of the phenotypic characteristics of known chitin-digesting *Vibrio* spp. with chitin-digesting organisms isolated from exoskeleton lesions of tanner crab (*Chionoecetes tanneri*) and dungeness crab (*Cancer magister*). Numbers in parentheses represent the total number of isolates tested.

Characteristic	Tanner crab (60)	Dungeness crab (22)	*V. parahaemolyticus* (44)	*V. alginolyticus* (23)	*V. anguillarum* (10)
Morphology	rod	rod	rod	rod	rod
Gram reaction	neg	neg	neg	neg	neg
Motility	60+	22+	44+	23+	10+
Growth in 1% Trypticase					
with 0% NaCl	8+	0+	0+	0+	0+
with 3% NaCl	60+	22+	44+	23+	10+
with 7% NaCl	52+	22+	44+	23+	8+
with 10% NaCl	8+	0+	0+	23+	0+
Growth at					
0°C	60+	0+	0+	0+	0+
25°C	30+	22+	44+	23+	10+
37°C	0+	0+	44+	23+	6+
Oxidase reaction	60+	22+	44+	23+	10+
Catalase reaction	60+	22+	44+	23+	10+
Luminescent	28+	0+	0+	0+	0+
Nitrate reduction					
to NO_2	52+	22+	43+	23+	10+
to NH_3	0+	0+	1+	0+	0+
Indole production	24+	22+	44+	23+	10+
Acetyl methyl carbinol production	28+	0+	0+	23+	0+
Citrate utilization	52+	22+	44+	23+	10+
Production of					
Lysine decarboxylase	30+	0+	44+	23+	0+
Arginine dihydrolase	52+	22+	0+	0+	10+
Amylase	30+	22+	44+	23+	10+
Lipase	32+	22+	44+	23+	10+
Gelatinase	32+	22+	44+	23+	10+
Carbohydrate utilization (acid only)					
Starch	32+	22+	44+	23+	10+
Sucrose	30+	22+	4+	23+	8+
Lactose	0+	0+	0+	0+	0+
Maltose	52+	22+	44+	23+	10+
Glucose	56+	22+	44+	23+	10+
Cellobiose	52+	22+	35+	19+	10+
Arabinose	30+	20+	33+	1+	8+
Mannitol	56+	22+	44+	23+	10+
Mellibiose	38+	0+	0+	0+	0+
Inositol	0+	0+	0+	0+	0+
Rhamnose	0+	0+	0+	0+	0+
Rapid chitin hydrolysis (3 d at 10°C)	28+	0+	0+	0+	0+

In general, the natural incidence of exoskeleton lesions on various species of crustaceans is quite low and rarely exceeds 10% of the population (Bright et al. 1960; Rosen 1967, 1970; Sindermann 1970). Highest incidence of lesions is correlated with increases in water temperature, fecal pollution, high levels of organic and inorganic wastes including carbohydrates and heavy metals, and crowding (Gopalin and Young 1975; Krantz et al. 1969; Nimmo et al. 1977; Young and Pearce 1975; Rosen 1970). Presumably these conditions increase the likelihood of epicuticle damage, which is considered to be a necessary prerequisite to lesion formation (Rosen 1970; Unestam and Weiss 1970).

The epicuticle of crustaceans is composed of polyphenolic compounds, which are presumably recalcitant to microbial attack (Dennell 1947). The very high incidence of bacteria on the epicuticle of undiseased specimens of *C. tanneri* substantiates this claim, particularly since no colonized bacteria were ever detected on the exoskeleton of crabs having intact epicuticles. It is thus presumed that the epicuticle must be damaged somehow before chitin-digesting bacteria can colonize and decompose the chitinous exoskeleton. Indeed, exoskeleton lesions have been transferred to various crustaceans in

TABLE 3. Some biochemical characteristics used to differentiate species of marine luminous bacteria. P is polar flagella; P and Pr refers to polar flagella in liquid medium and polar-peritrichous flagella on solid medium.

Characteristics	*Photobacterium phosphoreum*	*P. leiognathi*	*P. fisheri*	Luminous *Beneckea* sp. (*Vibrio* sp.)	Tanner crab isolates
Oxidase	−	+	+	+	+
Flagellation	P	P	P	P and Pr	P
Growth at					
4°C	+	−	−	−	+
25°C	+	+	+	+	− to slight
35°C	−	+	+	+	−
Voges-Proskauer	+	+	− (25%)[a]	−	+
Production of					
Lysine decarboxylase	+	+	+	+	+
Arginine dihydrolase	+	+	−	−	+ (slow)
Ornithine decarboxylase	−	−	−	+	+
Chitinase	+	+	+	+	+
Gelatinase	−	−	− (8%+)[a]	+	−
Amylase	−	−	−	+	−
Lipase	−	−	+	+	−
Utilization as sole source of C and energy					
Acetate	−	+	−	+	−
D-α-alanine	−	−	−	+	−
Cellobiose	−	−	+	+	+
Glucose	+	+	+	+	+
D-gluconate	+	+	−	+	+
D-glucuronate	(49% +)[a]	−	−	+	−
DL-lactate	(18% +)[a]	+	−	+	−
Maltose	+	−	+	+	+
Mannitol	−	−	+	+	+
L-proline	−	+	+	+	+
Propionate	−	−	−	+	−
Pyruvate	−	+	−	+	−
D-xylose	−	−	−	−	−

[a]Taken from Reichelt and Baumann (1973).

this manner (Cook and Lofton 1973; Unestam and Weiss 1970). However, occasional ruptures to the epicuticle due to mechanical damage would not be sufficient to account for the extensive necrosis noted on adult female *C. tanneri*. Slow degradation of the epicuticle by indigenous epiphytic bacteria may be the likely explanation for the extensive exoskeleton damage observed. Mechanical damage due perhaps to some aspect of mating behavior or to predator–prey relationships may also play a role in lesion formation. Interestingly, it was observed that the bacteria isolated from the epicuticle of *C. tanneri* were strictly oxidative and invariably capable of lipid hydrolysis. Similar organisms were also isolated along with chitinoclastic species from most lesions sampled. There is insufficient information at the present time to ascertain the significance of this observation regarding lesion formation.

Clearly the important conditions that influence the formation of exoskeleton lesions in most inshore animals do not exist in the deep waters traversed by the tanner crab. Therefore, a unique set of factors may be involved in lesion formation in this species. The problem, moreover, is that even under the optimum conditions for exoskeleton lesion formation as previously described, the incidence of inshore animals showing lesions have never approached the high incidence noted on adult tanner crabs. Obviously, the high percentage of infected animals and the high incidence of multiple lesions among female *C. tanneri* is due at least in part to the fact that females within the family *Majidae* cease to molt after the puberty molt (Pereyra 1972). The marked dimorphism, as measured by carapace widths, noted between adult female (102.5 mm) and male (148.9 mm) tanner crabs is an obvious result of this characteristic (Pereyra 1972). It is not known how long adult female tanner crabs live, but Ito (1970) estimated that the related species, *C. opilio*, can live from 3–4 yr after the maturity molt. The significant decrease in the incidence and severity of necrotic lesions on male tanner crabs is probably due to their ability to regularly molt, particularly since it has been reported that exoskeleton lesions are not transferred by ecdysis (Bright et al. 1960). This fact, however, does not explain why other majid crabs, such as the king crab (*P. camtschatica*), show considerably <10% incidence of necrotic lesions under in situ conditions (Bright et al. 1960) or why other species of crustaceans obtained at the same time *C. tanneri* was sampled did not show any signs of exoskeleton lesions.

Of the four species of *Chionoecetes*, extensive necrotic

lesions have only been observed on *C. tanneri*. Hyning and Scarborough (1973), however, reported that *C. bairdii*, which inhabits the shallow waters off Alaska, is commonly covered by a black encrustation that was found to be caused by the fungus *Phoma fimeti*. No mention was made as to whether or not exoskeleton lesions as found on tanner crabs were also observed on *C. bairdii*. *Phoma fimeti* or other fungal species were not isolated from tanner crab lesions.

The relationship between exoskeleton lesions and mortalities in the tanner crab was not investigated. Even though *C. tanneri* specimens were frequently found with severe lesions that extended through all of the layers of the exoskeleton, no clear relationship was seen between dead crabs and multiple lesions. As noted, most of the known crustacean pathogens are incapable of growth in the deep sea. This, of course, does not discount the possibility that the *Photobacterium* sp. isolated from lesions or some other deep-sea-adapted bacterial species could infect the hemolymph and cause mortalities. There are, for example, early reports that a luminous bacterium was pathogenic to the amphipod crustacean *Talorchestia longicornus* (Inman 1927). Infection of amphipods with pure cultures of this luminous organism resulted in death of the animals. Nothing is known of the immunological response of *C. tanneri* to bacterial invasion; however, Cornick and Stewart (1975) noted that the lobster pathogen, *A. viridans*, could remain viable in the hemolymph of the related crab species, *C. opilio*, for 100 d without any apparent pathogenic effect on the animal.

This is the first report of a *Photobacterium* sp. being the principal causative agent of exoskeleton lesions of deep-water crustacean species. Luminous bacteria have frequently been observed associated with crustaceans and with the gut contents, including fecal pellets, of fish that feed on these crustaceans (Harvey 1952; Raymond and DeVries 1976; J. A. Baross unpublished data). Most of these common free-living luminous bacteria are *Vibrio* spp. and although species of *Photobacterium* have been isolated from seawater, they are particularly known for their ability to enter into symbiotic associations with marine animals (Ruby and Nealson 1976). Some of these associations are quite important to the behavior of these animals and may involve prey–predator relationships, interspecies communication, and feeding behavior (Morin et al. 1975). There is no precedent in which a luminous bacterial species has unequivocally been shown to be associated symbiotically with a crustacean species. There are, however, some interesting features of the present study that indicate a possible association between *C. tanneri* and a *Photobacterium* sp. that is more complicated than just a simple infection.

Acknowledgments

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Distribution of Humpback Whales (*Megaptera novaeangliae*) in the Caribbean Determined by a Rapid Acoustic Method

COLEMAN LEVENSON AND WILLIAM T. LEAPLEY

Naval Ocean Research and Development Activity, NSTL Station, Miss. 39529, USA

LEVENSON, C., AND W. T. LEAPLEY. 1978. Distribution of humpback whales (*Megaptera novaeangliae*) in the Caribbean determined by a rapid acoustic method. J. Fish. Res. Board Can. 35: 1150–1152.

Humpback whale (*Megaptera novaeangliae*) distribution in the eastern Caribbean Sea was studied by means of solitary sonobuoys deployed from an oceanographic aircraft. Humpback whale sounds were detected on 33 of 82 sonobuoys deployed. It is estimated that 62 whales were detected in an area of 22 140 km^2 by this method with whale density calculated at 2.8×10^{-3} whales/km^2. For further experiments in selected areas we used a colinear array to obtain accurate counts of humpbacks. A total of 18 humpbacks were detected in this manner with a mean of 2.6 whales per array deployment. Combined acoustic and visual detections indicate a minimum of 119 humpbacks in the area surveyed. This rapid acoustic method could also be applied to detection of almost all odontocete cetaceans and to other marine mammals with distinctive vocal patterns.

Key words: cetaceans, whales, acoustic detection, distribution, density, humpbacks, aircraft detectors, sonobuoys

LEVENSON, C., AND W. T. LEAPLEY. 1978. Distribution of humpback whales (*Megaptera novaeangliae*) in the Caribbean determined by a rapid acoustic method. J. Fish. Res. Board Can. 35: 1150–1152.

On a étudié la répartition du rorqual à bosse (*Megaptera novaeangliae*) dans l'est de la mer des Caraïbes, à l'aide de bouées acoustiques solitaires installées grâce à un avion de recherche océanographique. Des sons émis par des rorquals à bosse ont été détectés par 33 des 82 bouées. On évalue à 62 le nombre de rorquals détectés de cette façon sur une superficie de 22 140 km^2; la densité été évaluée à $2{,}8 \times 10^{-3}$ rorquals/km^2. D'autres expériences menées dans des régions choisies utilisaient une distribution colinéaire pour déterminer le nombre exact de spécimens. Cette méthode a permis de détecter 18 rorquals, pour une moyenne de 2,6 spécimens par distribution. Des relevés acoustiques et visuels combinés ont permis de détecter au moins 119 rorquals à bosse dans la région inventoriée. Cette méthode acoustique rapide pourrait s'appliquer à la détection de presque tous les cétacés odontocètes ainsi que d'autres mammifères marins qui présentent un registre de voix distinctif.

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THE Naval Ocean Research and Development Activity (NORDA) is conducting studies to determine areal and seasonal distribution of large marine mammals. An airborne survey during January 1973 used acoustic techniques to acquire a quasi-synoptic view of humpback whale (*Megaptera novaeangliae*) distribution in the eastern Caribbean Sea. Knowledge of distribution and density of these whales in the Caribbean region is sparse. Slijper et al. (1964) indicate no humpback whales recorded by shipboard observers in this area for January, and <10 whales during February. Erdman (1970) and Erdman et al. (1973) report observations of humpbacks in the Puerto Rico–Virgin Islands area from 1966 through 1969. Visual and acoustic detections of humpback whales in this region were made from a specially equipped ship by Winn et al. (1975), and visual records of this species were obtained by E. D. Mitchell (Arctic Biological Station, Ste. Anne de Bellevue, Que., personal communication) near Navidad and Silver banks.

Humpbacks are well known for distinctive underwater vocalizations that are easily recognized by trained listeners (cf. Schevill and Watkins 1962; Winn et al. 1970; Payne and McVay 1971; Levenson 1972). Their acoustic output has been previously measured (Levenson 1972; Thompson et al. 1977). Broadband maximum and mean source levels are about 174 and 155

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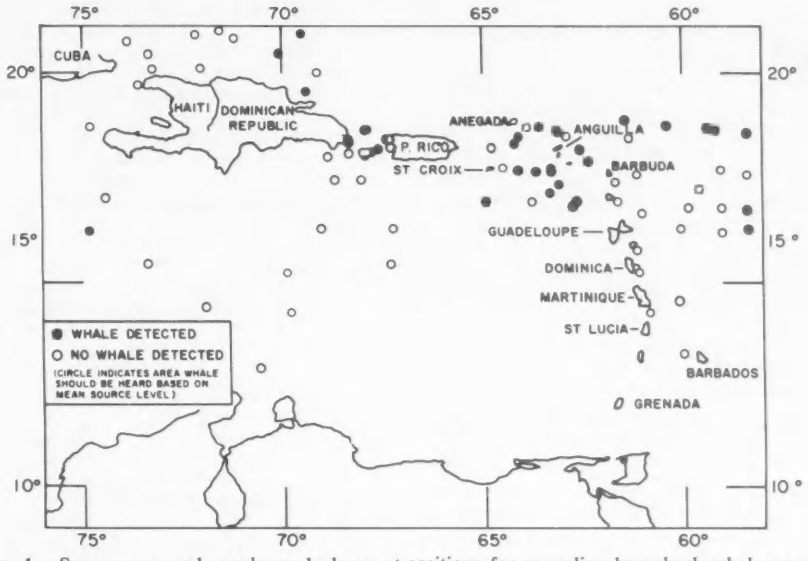

FIG. 1. Survey area and sonobuoy deployment positions for recording humpback whale sounds.

dB, respectively. Sounds are produced in a frequency range of from ~100 Hz to 8 kHz with most energy concentrated below 4 kHz.

Materials and methods — Predetermined flight tracks for this survey were flown by a Navy P-3A Orion, at 300 m altitude and an average speed of 370 km/h (200 knots). Passive SSQ-41 sonobuoys were deployed while a visual watch for whales was maintained. Sonobuoy hydrophones were set to operate at a depth of 20 m and were monitored for ~10 min after deployment. Data were recorded by a four-channel analog tape recorder. The recording system is described in a previous paper by Levenson (1974).

Results and discussion — Figure 1 shows the distribution of 82 sonobuoys deployed during six flights in late January 1973. Humpback whale sounds were recorded from 33 of these sonobuoys. With one exception, these positive detections occurred east of 70°W and north of 16°N. By simply listening to the tape recordings it can be determined that a single source was responsible for sounds relayed by 13 of these sonobuoys, while two or more whales appear to have contributed to vocalizations picked up by the other 20 sonobuoys. It is subjectively estimated that 62 whales were detected in this manner.

Taking the mean source level (155 dB re 1 μPa) and an ambient broadband noise level of ~75 dB re 1 μPa (Wenz 1962) we can, by assuming spherical spreading, draw a circle of probability of detection around a deployed sonobuoy with a radius of 9.3 km representing a mean area within which noise-emitting whales should be located. These circles of probable detection are shown in Fig. 2 for sonobuoys deployed in Mona Passage. By assuming each sonobuoy covers an area of

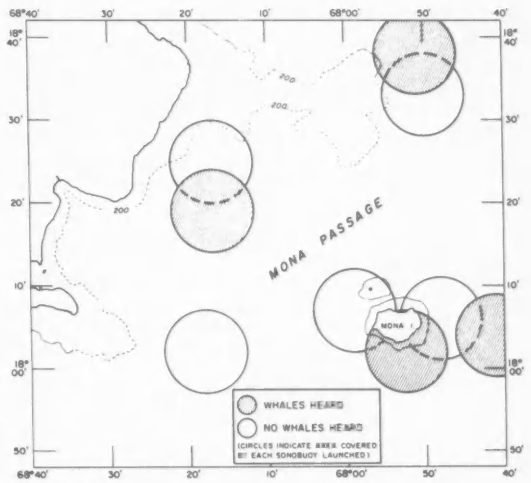

FIG. 2. Areas of probable detection from sonobuoy deployment in Mona Passage, off east coast of Dominican Republic.

270 km^2, we can estimate a total area of 22 140 km^2 covered by the 82 sonobuoys and a density of whales in the area surveyed of 2.8×10^{-3}/km^2. This compares to Winn et al. (1975, table 1 upper island chain) estimate of whales detected acoustically from a shipboard listening device of 3.2×10^{-3} whales/km^2 in an area of 12 745 km^2.

Since there is no reliable way to get an exact count of whales detected by a single sonobuoy, further experiments were conducted deploying three sonobuoys in a

colinear array as described by Levenson (1974). Differences in sound arrival times were used to determine the position of each sound source received by the array, thereby giving an accurate estimate of individuals present. On seven occasions during these flights when whales were observed, this multiple sonobuoy experiment was conducted. Analysis of recorded data from these events shows that 18 individual humpbacks were detected in this manner, with a mean of 2.6 whales per array deployment. This compared to the estimated 1.9 whales per contact subjectively determined by listening on single sonobuoy deployment. In addition to whales detected acoustically, trained observers maintaining a visual watch counted 53 whales observed where no sonobuoys were deployed. The combined acoustic and visual detections indicate a minimum of 119 humpbacks in the survey area. This compares with a total of 52–59 humpbacks (11 visual, 41–48 acoustic) counted by Winn et al. (1975) in the same general area.

These data show that combined acoustic and visual detection from aircraft offers a rapid method of determining marine mammal distribution. With this approach, qualitative synoptic population distribution studies can be conducted. In addition, quantitative estimates of marine mammals with distinctive vocal patterns can be achieved by deploying multiple sonobuoy arrays in selected areas.

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A Device to Simulate the Variability in Sunlight Intensity for Laboratory Cultures of Algae

CARL M. BOYD AND JOHN MARRA[1]

Department of Oceanography, Dalhousie University, Halifax, N.S. B3H 4J1

BOYD, C. M., AND J. MARRA. 1978. A device to simulate the variability in sunlight intensity for laboratory cultures of algae. J. Fish. Res. Board Can. 35: 1152–1154.

Simulation in the laboratory of the variability of environmental light intensity is achieved by automatic continuous control of voltage applied to a tungsten light source.

Key words: light intensity, variable light, photosynthesis, diurnal rhythms

BOYD, C. M., AND J. MARRA. 1978. A device to simulate the variability in sunlight intensity for laboratory cultures of algae. J. Fish. Res. Board Can. 35: 1152–1154.

On a pu, grâce à un contrôle continu automatique de tension appliqué à une source lumineuse au tungstène, simuler au laboratoire la variabilité de l'intensité lumineuse de l'environnement.

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[1]Present address (J.M.): Lamont-Doherty Geological Observatory, Palisades, N.Y. 10964, USA.

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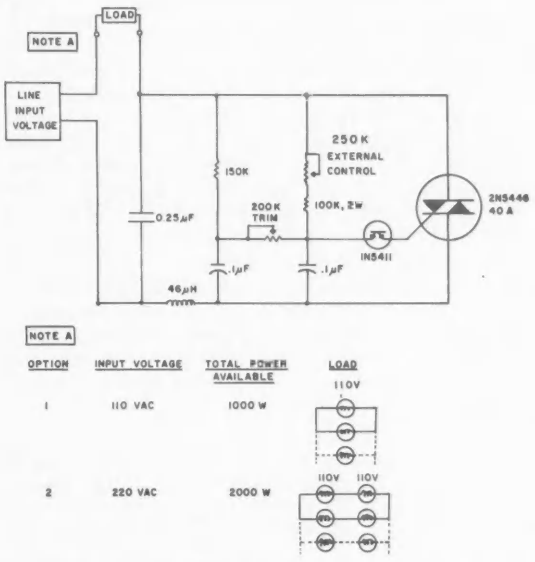

FIG. 1. Circuit diagram for simulation device.

A common problem in laboratory studies of algal growth and photosynthesis is the reproduction, under controlled conditions, of the natural light environment in terms of intensity and color variations. We describe here a device that simulates sunlight intensity variations by controlling the voltage applied to a tungsten filament lamp on a predetermined program. Although we have not solved the problem of simulating the variation in spectral quality of underwater light, the technique allows considerable latitude in the choice of color correction and adds another element of manipulation to the experiments of physiologists. The device has been employed in two research projects in this laboratory using laboratory cultures of marine algae and has proven easy to use. Other techniques for achieving diurnal variation of light intensity in the laboratory include the use of sequence timers to control light banks (Glooschenko et al. 1972; Woodring 1968) and the use of motor-driven rotating shutters in the light path (Quraishi and Spencer 1971). Stewart (1963) described a device similar to ours in that intensity was governed by a cam; the electronics associated with that device were relatively expensive compared with our device which incorporates simplicities that derive from advances in solid-state physics. The advantages of the device described here are simplicity and flexibility in simulating either diurnal variation, short-term fluctuations (Marra unpublished data) or light regimes resulting from coherent movements in the euphotic zone (see Kamykowski 1973). Also, as it is currently being used, the equivalent of noon sunlight intensities (ca. 50 $mW \cdot cm^{-2}$) can easily be attained.

The electronic circuit is shown in Fig. 1; components

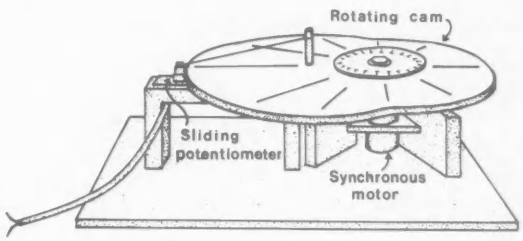

FIG. 2. External control unit (cam, timer, and sliding potentiometer); the cam shown here is designed for simple diurnal variation.

can be purchased for about $150 and can be easily assembled. The circuit is a standard Triac controlled power supply with the addition of the two electronic noise suppression filters (the 0.25-μF capacitor and the 46-μH, 20-A coil). The 200-K trimpot permits adjustments to prevent the tungsten filament from going off completely and thus facilitates gradual turn-on of the bulb at the beginning of the photoperiod.

The voltage variation is accomplished by the external control (Fig. 2) which consists of a common 24-h timer (we use Intermatic Time-all Model A921-6), a cam (0.64-cm-, or 0.25-in.-, thick polyvinyl chloride), and a 250-K sliding potentiometer (7.5 cm, or 3 in., long from Slideline, Duncan Electronics, Costa Mesa, Calif.). The timer rotates the cam which in turn controls the position of the potentiometer and therefore the voltage applied to the lamp. To ease the friction between the moving cam and the control stub of the potentiometer, the cam edge was smoothed and given a light coat of silicone stopcock grease. In addition, a short piece of polyethylene tubing was placed over the control stub. An elastic band kept the control stub against the cam on decreasing light intervals.

A second version of the apparatus likewise employs a cam (mounted on an Intermatic T103 timer) to control a sliding potentiometer that is part of a dimming switch available for household lighting (Lutron, Coopersburg, Pa.). The short sliding travel of the potentiometer (16.5 mm, or 0.650 in.) built into this dimmer imposes more stringent requirements on the machining of the cam than is necessary when the 7.5-cm sliding potentiometer and associated electronics are employed. However, the lesser expense ($30) and the ease of assembly (the sliding dimmer replaces all the Triac electronics and the potentiometer) make this version desirable for many applications.

The cams were produced from calibrations of the electronics with the light source in place. Photosynthetically active radiation incident on the culture vessel surface was determined for several positions on the sliding potentiometer, producing a (nonlinear) curve of light intensity vs. distance. Intensity was measured with a LI-185 quantum Radiometer-Photometer (Lambda Instruments, Lincoln, Nebr.), using the photosyntheti-

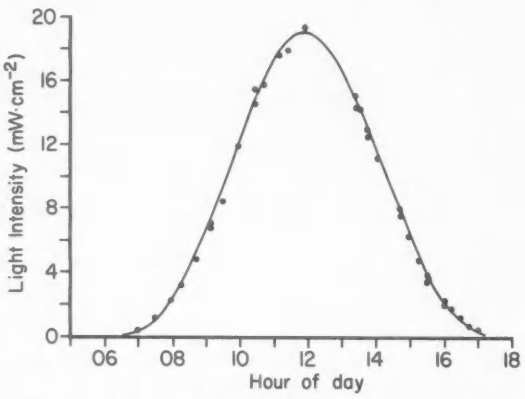

FIG. 3. Light intensity curve. Solid line represents an example of a desired light intensity program based on Ikusima's formulation; points represent the achieved light intensity (in milliwatts per square centimetre) incident on the phytoplankton incubation chamber.

cally active radiation (PAR) sensor that measures quanta in a bandwidth of 400–700 nm. For cams simulating diurnal variation, this curve was combined with Ikusima's (1967) equation for the variation in sunlight intensity for a fine day,

$$I(t) = I_{max} \sin^3 (\pi t/\lambda),$$

where $I(t)$ is the light intensity at time t, I_{max} is the noon intensity, and λ the day length with the origin at the beginning of the photoperiod (Fig. 3). The cam can then be modeled on the basis of sliding potentiometer distance vs. time of day. Deviations from the daily light pattern can be accomplished by simply bolting on a differently designed cam. We have built and used cams with as many as seven cycles of light variation; very complex programs of variation of intensity may be developed simply by shaping the cam in the appropriate manner.

We operate the device on 110 VAC with one General Electric 500-W quartz-iodine spotlight (Q500-PAR/NSP) or on 220 VAC with two spotlights in series. Only one light is required to produce the desired intensity; the second unit may be used for duplicate experiments. Figure 1 indicates the lighting options; additional light sources can be employed on separate cultures or can be configured to change the directional source of light impinging on the culture. The light beam as we normally use it passes through a two-compartment filter: tap water (4 cm thick) flowing to drain in the first compartment and 2% solution of $CuSO_4 \cdot 5H_2O$ (1 cm thick) in the second. Both the cooling water and the cupric sulfate solution remove infrared energy, thereby altering the tungsten source spectrum to give a peak of about 600 nm. The cupric sulfate solution also dampens the spectral shift toward the red as voltage to the lamp is reduced. Noticeable spectral shifts occurred only in the first and last hours of the photoperiod, which is at least qualitatively similar to what happens in nature. This filter was chosen because of its simplicity and because it does not cause large losses in intensity. There are, however, other filter types that could be easily employed. Crawford and Nimeroff (1968) and Bickford and Dunn (1972) have reviewed methods to reproduce the natural light spectrum at the sea surface, and Davis et al. (1973) and Bhovichitra and Swift (1977) have recently described light filters to approximate the quality of underwater light.

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Acoustic Detection of Demersal Fish Closer than 15 Centimetres to the Bottom in 80 Metres of Water

M. H. ORR, E. E. HAYS, AND F. R. HESS

Woods Hole Oceanographic Institution, Woods Hole, Mass. 02543, USA

ORR, M. H., E. E. HAYS, AND F. R. HESS. 1978. Acoustic detection of demersal fish closer than 15 centimetres to the bottom in 80 metres of water. J. Fish. Res. Board Can. 35: 1155–1156.

Records from an acoustic backscattering system operating at 200 kHz are presented which show that demersal fish can be individually detected closer than 15 cm to the ocean bottom in 88 m of water.

Key words: acoustic, demersal fish

ORR, M. H., E. E. HAYS, AND F. R. HESS. 1978. Acoustic detection of demersal fish closer than 15 centimetres to the bottom in 80 metres of water. J. Fish. Res. Board Can. 35: 1155–1156.

Nour présentons dans cet article les enregistrements d'un système de rétrodiffusion acoustique fonctionnant à 200 kHz, qui démontrent que l'on peut détecter individuellement des poissons démersaux à moins de 15 cm du fond de l'océan dans 88 m d'eau.

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ACOUSTIC systems are routinely used to detect the presence and estimate the biomass of pelagic fish (Cushing 1973). Due to their proximity to the bottom, demersal fish are more difficult to detect as the acoustic signal that is reflected from them often merges with the bottom reflected signal. An acoustic system operating at 36 kHz with a 25-cm resolution capability has been used to count demersal fish within 3.8 m of the bottom (Dowd 1967). Higher acoustic frequencies (e.g. 200 kHz) have the theoretical ability of attaining higher resolution and thus better separation of individual demersal fish from the ocean bottom.

Recently, a research cruise was undertaken in Massachusetts Bay (R.V. *OCEANUS 32*) to perform an interdisciplinary study of the short period internal waves which are known to propagate into the bay with each flood tide. An acoustic system of high sensitivity was used to detect the internal wave fields. A portion of the acoustic experimental period was used to determine whether the acoustic backscattering system could detect and resolve demersal fish that were close to or on the bottom.

Experiment — A sensitive broadband acoustic backscattering system has recently been designed and constructed at the Woods Hole Oceanographic Institution. Two transducers are housed in a flow body. The flow body is towed from a boom extending 4.6 m to the side of the ship. The normal tow depth was about 1 m. One transducer, which is broadband and covers the range of 15–45 kHz, was operated at 40 kHz with a pulse length of 500 μs. Its full beamwidth at the half value angle (e.g. see Forbes and Nakken 1972, p. 12, for definition of terms) was 7°. The second transducer, full beamwidth of 2.5° at the half value angle, was operated at 200 kHz with a pulse length of 100 μs (7.5-cm resolution capability).

An EPC 4600 graphic recorder and a Honeywell Fibre Optics Cathode Ray Tube Visicorder Oscillograph Model 1856A, which allowed the production of high resolution records, were used to generate the graphics displays (Fig. 1 and 2).

Data — The presence of demersal fish within 1–2 m (Fig. 1a, arrow 1) of the bottom is revealed by the 40-kHz data. The hyperbolic traces from individual fish merge with each other and/or the bottom (arrow 2) creating confusion if a total body count is to be made.

The 200-kHz narrow beam system, however, resolved individual fish (Fig. 1b, arrow 1). Some were less than 1 m from the bottom (arrow 2). At a depth of 88 m the beam of the 200-kHz acoustic system interacts with a bottom section 3.84 m diam. The diffuse cloud of scatterers in the 45–75-m range was found to consist of a variety of amphipods and euphausiids. The Honeywell Fibre Optic Recorder was used to produce a display of the 200-kHz acoustic signal received from the lower 11 m of the water column (a portion is presented, Fig. 2). Arrow 1 (Fig. 2) points to a group of four individuals which were detected to within 2 m of the bottom. Fish were detected to within 10 cm of the bottom (arrow 2). There are discontinuities closer than 10 cm to the

[1]Woods Hole Oceanographic Institution Contribution No. 4070.

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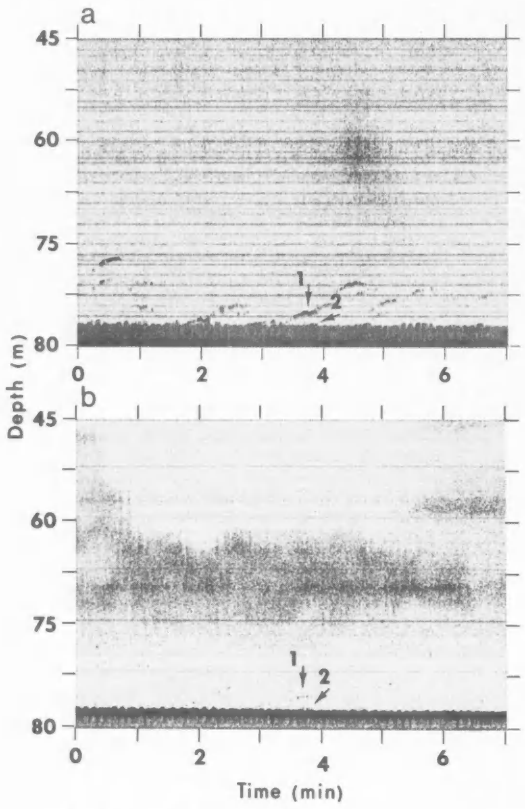

FIG. 1. Section of a split screen display of 40- (a) and 200-kHz (b) data obtained during the same time interval from a drifting ship. Water depth was 88 m. The 40-kHz system, due to the larger beamwidth of the transducer, detected more fish than the 200-kHz system.

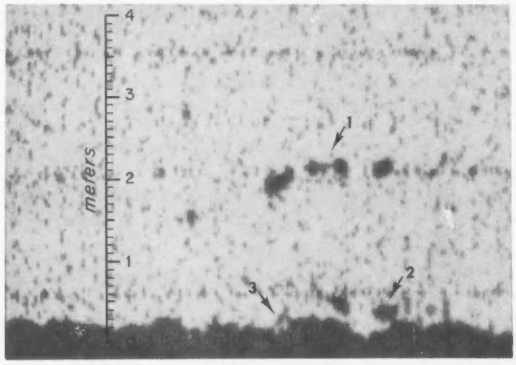

FIG. 2. High speed recording of 200-kHz data showing individual demersal fish to within 10 cm of the ocean floor (arrow 2). The presence of scatterers closer than 10 cm to the bottom are observed in the record (arrow 3).

bottom (arrow 3) which are probably also demersal fish. The fish were observed to leave the proximity of the bottom and migrate toward the surface with the onset of the evening twilight. This is a characteristic of the cod, haddock, and hake which are commonly caught in these waters.

Acknowledgments— This work was partially supported by the National Science Foundation Grant No. OCE77-08682, by NORDA Contract No. N00014-77-C-0196, and NOAA Contract 04-6-158-40072.

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LETTERS/LETTRES

Comment on "Food of Some Benthic Harpacticoid Copepods"

COMMENT

Brown and Sibert (1977) claim that heterotrophically (i.e. bacteria) derived carbon was ingested by harpacticoid copepods at a rate 9 times faster than autotrophically (i.e. algae) derived carbon, and that the difference in assimilation rates is due to differential uptake. In this note I would like to discuss several unexplained points in their conclusions.

The composition of the carbon sources of the collected material described as "surface sediments" is not given at any stage of their experiments. It could be organic particles, bacteria, benthic invertebrates, protozoans, algae, and others, since organisms other than algae and bacteria can multiply in the enriched incubated sediments. Moreover, small invertebrates can utilize labeled bacteria and algae during the described period of incubation and serve as a food source for the harpacticoids. The organic particles of the sediments, which have a large, free surface area, can adsorb dissolved, labeled substances and bacteria and be raked in by the copepods. Harpacticoid mouth parts are adapted for raking, seizing, and scraping food particles (Pennak 1953) larger than bacteria. Therefore, thriving of harpacticoids when fed on algae or bacteria is suggested to be partly due to the formation of algal and bacterial clusters which sink to the bottom and are scraped up by the harpacticoids and not by sieving suspended cells. Cluster formation and food particle sizes should be taken into account when analyzing the results of harpacticoid feeding experiments.

Brown and Sibert measured the radioactivity of the carbon collected on filters, and stated that the carbon that was utilized by the harpacticoids came from algal and bacterial sources. How can the authors make such a statement when microscopical identification of the material collected on the filters was not done?

The authors claimed that assimilation was equal to food intake in their experiments, but without measurements of radioactivity of the fecal pellets. Such a rare situation can take place only when the digestion coefficient is 100% when food quality is very high. Usually there is an equilibrium between ingestion, assimilation, and defecation which is strongly affected by environmental conditions (McMahon and Rigler 1963; Winberg 1971; Gophen 1976, 1977). In comparison with ingestion rate, the rate of assimilation is lower and the process of assimilation begins later than ingestion. Therefore, assimilation experiments should be carried out with longer than 0.5–6.0 hours exposure times as used by Brown and Sibert. Chase-type experiments, for example, could improve assimilation measurements.

In the autotrophic food source it was stated that there were large benthic diatoms. A high portion of these diatoms, the frustules, is undigestible material and is defecated. This undigested, probably labeled material will lower the assimilation coefficient associated with a higher amount of defecated material in comparison with feeding on bacteria (Gophen et al. 1974; Gophen 1977) or on organic particles.

In dark-incubated material, labeled glucose can be heterotrophically utilized either by bacteria or algae (Berman et al. 1977), different invertebrates, or adsorbed onto organic particles. I think that in the dark-incubated material the harpacticoids ingested labeled food with a higher assimilation coefficient and not higher ingestion compared with the light-incubated food (containing more algae). Consequently defecation was reduced and the incorporation of radioactive material increased when dark-incubated food was utilized. There were differences in assimilation rates that are not due to differential uptake as the authors concluded.

To study the feeding habits of zooplankton organisms on two different food sources, a better method would be to compare ingestion and assimilation rates (long and short exposure times) when separate and mixed food sources (microscopically identified) are given (Gophen et al. 1974; Lampert 1974; Gophen 1977). — M. GOPHEN, *Israel Oceanographic and Limnological Research Ltd., Kinneret Limnological Laboratory, P.O.B. 345 Tiberias, Israel.*

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REPLY

Dr Gophen makes several points in his letter to which we will reply. First, Dr Gophen correctly points out that many organisms other than bacteria and algae are capable of multiplying in enriched sediments and of consuming labeled algae or bacteria. In our experiments, the food sources were in contact with the labeled compounds for only 12 hours. While some of the label may have found its way into higher trophic levels during this period, most of it was probably in the primary (i.e. heterotrophic or autotrophic) levels. This question could be settled by microautoradiography although we have not done that experiment. The issue is not particularly important to our conclusions. The heterotrophic carbon source (i.e. ^{3}H-glucose) found its way into the harpacticoids faster than the autotrophic carbon source (i.e. ^{14}C). Since we are interested in the role of dissolved organic carbon in estuarine ecosystems, it is not even particularly important whether the heterotrophy involved is due to algae or bacteria. The results of Munro and Brock (1968), however, show that algal heterotrophy is negligible compared to bacterial heterotrophy.

Second, Dr Gophen suggests that harpacticoids are adapted to feed on particles larger than bacteria. More recent and detailed studies of the functional morphology and behavior of harpacticoids (e.g. Marcotte 1977) have shown that harpacticoids have a great diversity of adaptations for feeding on bacteria as well as on other particles.

Third, we agree with Dr Gophen on the importance of particle size and food clusters. We are currently preparing some experiments to explore this problem.

Fourth, Dr Gophen suggests that we claimed that assimilation was equal to uptake in our experiments. We made no such claim. We did claim that the observed increase in activity in the copepods represented assimilation rather than uptake because of the length of the experiments and the production of fecal pellets. We did not determine the activity in the fecal pellets and we have no estimate of respiration and therefore we do not know the actual uptake rate. We should point out that our experiments were similar in design, length, and conclusion to those reported by Gophen et al. (1974). Dr Gophen further suggests that a high portion of diatom tissue may not be digestible and the assimilation coefficient may therefore be decreased. We agree with this speculation but fail to understand why harpacticoids would not also discriminate against such undigestible food particles. Net assimilation rate must be a function of both uptake rate and assimilation efficiency.

Finally we would like to point out that estuarine benthic harpacticoid copepods do not resemble zooplankton in very many ways. Unlike zooplankton, these organisms are accustomed to living in intimate association with sediments and other particles, on surfaces, and on edges. Such environments have large and diverse standing crops of both bacteria (Sieburth 1975) and organic detritus. It should not be surprising therefore if heterotrophically processed food sources turn out to be the principal foods of benthic harpacticoids. — J. R. SIBERT AND T. J. BROWN, *Department of Fisheries and the Environment, Fisheries and Marine Service, Pacific Biological Station, Nanaimo, B.C. V9R 5K6.*

GOPHEN, M., B. Z. CAVARI, AND T. BERMAN. 1974. Zooplankton feeding on differentially labelled algae and bacteria. Nature 247: 393–394.

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Comment on Book Review — "Retinas of Fishes. An Atlas"

COMMENT

It is our feeling that Dr Stell was not a suitable reviewer for this book. He is basically a neurocytologist of the retina who dwells in details of synaptic connections in the goldfish retina. Although he is competent in his field, he has little or no concept of comparative or ecological approaches to fish vision as emphasized in the book. If the reviewer had read the preface more carefully, he would have realized that the book was not addressed to him and that his criticisms of details were irrelevant.

The book is intended for ichthyologists, comparative anatomists, and sensory or vision physiologists as a source of information. Assembling materials from many locations and diverse colleagues leads inevitably to inconsistent quality. We knew the risks of such an undertaking. The reviewer would have us give more detailed descriptions of techniques used for each material; but the kind of prospective reader we envisaged would find no use in these and might even find it cumbersome. The reviewer contends that some descriptions are uncritical, vague, or misleading. The fact is, for many of those species there is little unequivocal information available and our text simply reflects that situation. On the other hand, if the curiosity of the reader is stirred, the book provides him with the means to dig further. We agree with the reviewer that the literature is incomplete; this was not intended as the final scholarly work on the subject, and mentions of the "classic 19th century works" were judged irrelevant to our immediate purpose. Besides, all these references are given in an historical review which is cited. Evidently, we are criticized for not mentioning recent neurocytological works dear to the reviewer, which dwell on specialized material and consequently would not be immediately useful to our prospective reader.

We regret that the reviewer took it as a personal insult

not to find in the book what he, as a specialist, expected to get. We do not believe, though, that his bitterness will disturb the discerning reader. It is a pity that when so many competent fish biologists interested in visual ecology, phylogeny, and histology are available, even in Canada, the book was sent to a neurocytologist affiliated to a medical institution, who lacks an overall view of the Atlas' subject. — M. A. ALI AND M. A. ANCTIL, *Département des Sciences Biologiques, Université de Montréal, Case postale 6128, Succursale "A", Montréal, Qué. H3C 3J7.*

STELL, W. K. 1977. Book review of: M. A. Ali and M. Anctil, 1977. Retinas of fishes. An atlas. J. Fish. Res. Board Can. 34: 2232–2233.

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REPLY

I agree with Professors Ali and Anctil that another opinion of their work should be heard and am pleased that they have submitted their own response for consideration. I, too, am certain that there are in Canada many competent fish biologists who know good work when they see it. I should like to hear what they have to say.

In response to the authors' criticism I must in all honesty confess that I am indeed a professional retinal neurocytologist; that I am affiliated to a medical institution; and that I do study the synaptic organization of fish retinas, with particular reference to ecologically and ethologically trivial features including rod and cone pathways, color vision mechanisms, and other structure/function correlations. Doubtless I was seduced by the book's title, as well as by statements in the Preface that among its purposes are "to assist the visual physiologist" and to provide "a ready pool of information for laymen, students, and specialists," into believing that it was intended for me. Thank heaven I am not the Generalist to whom the book is addressed. I should then take offense at the implication that technical details are beyond my ken; that whether it is the primary data cited or the text citations themselves that are "equivocal" should not concern me; that I should be steered away from "irrelevant" classical and recent publications on fish retinas and presented pages and pages of empty space instead.

No, indeed. Fortunate to be the specialist, I feel neither insulted nor bitter over the material presented in Retinas of Fishes. I simply do not believe it meets acceptable modern standards of scholarship or descriptive histology suitable for the ichthyologist, comparative anatomist, or sensory or visual physiologist. The authors, men of good investigative reputation and output, certainly must be judged capable of presenting a more profound and enlightening account. That they have not chosen to do so means that every reader, fish biologist, and retinal neurocytologist alike will find this work to be lean fare at banquet prices. — W. K. STELL, *Jules Stein Eye Institute, UCLA School of Medicine, Center for Health Sciences, Los Angeles, Calif. 90024, USA.*

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CORRECTIONS TO RECENT PUBLICATIONS
CORRECTIONS SUR RÉCENTES PUBLICATIONS

VOLUME 32, No. 12 — review by J. C. Davis: *Minimal dissolved oxygen requirements of aquatic life with emphasis on Canadian species: a review.*

Page 2297: Right column, par. 4, line 1, "At 19 C fresh water contains" should be "At 10 C fresh water contains".

Page 2325: Table 10. The percentage saturation values at 15 and 20 C are reversed for B level criteria for "Freshwater mixed fish population including salmonids". B level percentage saturation values should be 54 54 54 54 57 63.

VOLUME 32, No. 12 — paper by W. H. Lear: *Evaluation of the transplant of Pacific pink salmon (Oncorhynchus gorbuscha) from British Columbia to Newfoundland.*

Page 2353: Right column, par. 3, line 4, "pink salmon" should be "sockeye salmon"; Right column, par. 3, line 6, "22% increase" should be "220% increase."

Page 2354: Right column, par. 1, lines 2 and 3, "Vernon (1958)" should be "Gilhousen (1962)".

The following reference was not included in the reference section:

GILHOUSEN, P. 1962. Marine factors affecting the survival of Fraser River pink salmon, p. 105–111. *In* Symp. on Pink Salmon. H. R. MacMillan Lectures in Fisheries, Univ. British Columbia, Vancouver, B.C.

VOLUME 33, No. 5 — note by W. H. Lear: *Migrating Atlantic salmon (Salmo salar) caught by otter trawl on the Newfoundland continental shelf.*

Page 1204: Right column, par. 3, line 11, "consumption" should be "composition".

VOLUME 33, No. 5 — paper by W. S. Hoar: *Smolt transformation: evolution, behavior, and physiology.*

The following references were not included in the reference section:

ANON. 1963. UBC scientists mature salmon in fresh water. West. Fish. 67(1): 42.

BAGGERMAN, B. 1960. Salinity preference, thyroid activity and seaward migration of four species of Pacific salmon (*Oncorhynchus*). J. Fish. Res. Board Can. 17: 295–322.

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BERT, P. 1871. Sur les phénomènes et les causes de la mort des animaux d'eau douce que l'on plonge dans l'eau de mer. C.R. Acad. Sci. Paris 73: 382–385.

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CHIN, Z., AND K. KURODA. 1935. Sur l'élevage du saumon (*Oncorhynchus keta* Walbaum) pendant une longue durée dans l'eau douce. Keijo J. Med. 6: 30–40.

VOLUME 33, No. 6 — paper by M. Sinclair, M. El-Sabh, and J.-R. Brindle: *Seaward nutrient transport in the lower St. Lawrence estuary.*

In estimating seaward nutrient transport from the St. Lawrence estuary, nutrient concentrations were converted from mg-at/m^3 to tons/m^3 (and subsequently to tons/mo in the transport estimates) without multiplying by the appropriate atomic weight (Fig. 5, Table 2). This led to an underestimate of surface seaward nutrient transport by a factor equal to the atomic weight (for example 14 times for nitrogen).

VOLUME 34, No. 5 — note by N. P. Boyce and S. B. Yamada: *Effects of a parasite, Eubothrium salvelini (Cestoda: Pseudophyllidea), on the resistance of juvenile sockeye salmon, Oncorhynchus nerka, to zinc.*

Page 707: Left column, par. 3, line 7, "with" should be "to".

Page 709: Right column, Smith and Margolis (1970), "J. Parasitol. 56: 321–322." should be "J. Parasitol. 56 (Sect. II): 321–322."

VOLUME 34, No. 8 — paper by W. T. Momot and H. Gowing: *Response of the crayfish Orconectes virilis to exploitation.*

Page 1218: Table 6, data for North Twin Lake 1968 should be

Age-group		Age-group	
0	17.7	0	17.7
1	40.7	1	27.2
2	3.5	2	4.8
3	2.6	3	0.2
	64.5		49.9

VOLUME 34, No. 9 — paper by G. A. Jackson: *Nearctic and Palaearctic Paracladopelma Harnisch and Saetheria n.gen. (Diptera: Chironomidae).*

Page 1357: Acknowledgments, insert "Mr J. K. Hiltunen, U.S. Fish and Wildlife, Ann Arbor, Mich."

VOLUME 34, No. 11— paper by W. T. Momot and H. Gowing: *Production and population dynamics of the crayfish Orconectes virilis in three Michigan Lakes.*

Page 2049: Table 6, total ♂ + ♀ (kg) for North Twin Lake should be 125.7 not 124.8.

Page 2053: Table 8, the P:$\bar{B}$ ratio for North Twin Lake in 1967 should be 1.23 and in 1968 should be 1.35.

VOLUME 34, No. 11 — paper by W. T. Momot and H. Gowing: *Results of an experimental fishery on the crayfish Orconectes virilis.*

Page 2063: Table 6, the P/$\bar{B}$ ratios for North Twin Lake in 1967–68 should be 1.23; the 1968–69 data for North Twin Lake should be

$\bar{B}$	P	P/$\bar{B}$
49.0	66.2	1.35

VOLUME 34, No. 11 — paper by P. M. Ryan and H. H. Harvey: *Growth of rock bass, Ambloplites rupestris, in relation to the morphoedaphic index as an indicator of an environmental stress.*

Page 2081: Left column, par. 1, line 5, "coordinated" should be "coordinate".

Page 2082: Table 2, boxhead, "Mean total length (mm) at end of life in age-group" should read "Mean calculated total length (mm) at end of last full year of life of fish in age-group"; for La Cloche age-group 5 omit ±; for Florence age-group 4 read

95.8
4.1
(15)

VOLUME 35, No. 1 — paper by J. C. Sharp, K. W. Able, W. C. Leggett, and J. E. Carscadden: *Utility of meristic and morphometric characters for identification of capelin (Mallotus villosus) stocks in Canadian Atlantic waters.*

Page 125: Left column, Materials and Methods, par. 1, lines 9 and 10, "All morphometric measurements were made immediately after the fish had thawed." should read "Specimens were thawed, fixed in 10% formalin for approximately 1 wk, and then washed 1–2 h with tap water before taking morphometric measurements."

COMING NEXT MONTH/À PARAÎTRE LE MOIS PROCHAIN

Fecundity of witch flounder (*Glyptocephalus cynoglossus*) from St. Pierre Bank and the Grand Bank of Newfoundland **W. R. Bowering**

Myxosoma cerebralis: detection of circulating antibodies in infected rainbow trout (*Salmo gairdneri*) **B. R. Griffin and E. M. Davis**

Acute effects of underwater construction blasting on fishes in Long Point Bay, Lake Erie **G. C. Teleki and A. J. Chamberlain**

Revision of the turbellarian genus *Ectocotyla* (Seriata, Monocelididae) associated with the crabs *Chionoecetes opilio* and *Hyas araneus* **L. C. Fleming and M. D. B. Burt**

Zooplankton sample variation on the Scotian Shelf **D. D. Sameoto**

Summer phytoplankton variability in the lower St. Lawrence estuary **M. Sinclair**

Life history and abundance of *Mysis relicta* in Lake Michigan **M. D. Morgan and A. M. Beeton**

Notes

Rapid method for determining concentrations of Bayer 73 in water during lampricide treatments **V. K. Dawson, P. D. Harman, D. P. Schultz, and J. L. Allen**

Lag adjustment between estimated and actual physiological responses conducted in flow-through systems **A. J. Niimi**

Precocious sexual maturation in Atlantic salmon (*Salmo salar*) postsmolts reared in a seawater impoundment **A. M. Sutterlin, P. Harmon, and B. Young**

ANNOUNCEMENTS / AVIS

5th Annual Aquatic Toxicity Workshop

Call for Papers

This workshop will be held at the Royal Connaught Hotel, Hamilton, Ontario, November 7–9, 1978. It will be hosted by the Canada Centre for Inland Waters.

The purpose of the workshop is to provide a forum for discussions of issues relevant to aquatic toxicology; descriptions of new concepts, approaches, and methodology; critical reviews of "the state of the art"; presentation of new results of monitoring and research on toxic substances; and contacts among participants from universities, industries, and federal and provincial governments.

Papers for the program are solicited from the following program areas: Canada's environmental laws and aquatic toxicology; waste treatment effects on toxicity and mixed effluent studies; water quality objectives; and new techniques in bioassays. Abstracts (approximately 500 words) should be sent to the organizers by September 1, 1978. A detailed program will be available by October 1, 1978.

Please send the detailed abstracts and requests for further information to

Dr P. V. Hodson, Chairman of the Organizing Committee
Great Lakes Biolimnology Laboratory
Canada Centre for Inland Waters
867 Lakeshore Road
P.O. Box 5050
Burlington, Ontario
L7R 4A6
Telephone (416) 637-4559

5e Atelier de travail annuel sur la toxicité aquatique

Appel d'articles

Cet atelier de travail se tiendra à l'hôtel Royal Connaught, à Hamilton, Ontario, du 7 au 9 novembre 1978. Le Centre canadien des eaux intérieures en sera l'hôte.

L'atelier de travail fournira une tribune pour la discussion de questions touchant la toxicologie aquatique; la description de nouveaux concepts, approches et méthodologie; les revues critiques de l'état de la connaissance; la présentation de nouveaux résultats de la recherche sur les substances toxiques et leur surveillance; et enfin, les contacts parmi les participants des universités, des industries et des gouvernements fédéral et provinciaux.

On demande des articles dans les domaines suivants du programme : législation canadienne et toxicologie aquatique; effets du traitement des eaux usées sur la toxicité et études sur les effluents mixtes; objectifs de qualité des eaux; nouvelles techniques d'analyses biologiques. On devra faire parvenir aux organisateurs, avant le 1er septembre 1978, un résumé des articles (environ 500 mots). Le programme détaillé sera prêt le 1er octobre 1978.

On est prié d'adresser les résumés détaillés et les demandes de renseignements supplémentaires au

Dr P. V. Hodson, président du Comité d'organisation
Laboratoire de biolimnologie des Grands Lacs
Centre canadien des eaux intérieures
867 Lakeshore Road
Case postale 5050
Burlington, Ontario
L7R 4A6
Téléphone (416) 637-4559

SI Units and Symbols

We encourage use of SI units (Système international d'unités) and symbols in the *Journal of the Fisheries Research Board of Canada* for the following list of quantities. A more complete list is given in the *Canada Metric Practice Guide* (CAN3-Z234.1-76) available from the Canadian Standards Association, 178 Rexdale Blvd., Rexdale, Ontario M9W 1R3.

Unités et symboles SI

Nous encourageons l'usage des unités SI (Système international d'unités) et des symboles pour le *Journal de l'office des recherches sur les pêcheries du Canada* pour la liste des quantités ci-dessous. Le *Guide canadien de familiarisation au système métrique* (CAN3-Z234.1-76), que l'on peut se procurer auprès de l'Association canadienne de normalisation, 178, boul. Rexdale, Rexdale (Ontario) M9W 1R3, donne une liste plus complète des unités.

Quantity/Quantité	Name/Nom	Symbol/Symbole
length/longueur	centimetre, kilometre . . ./centimètre, kilomètre . . .	cm, km . . .
area/aire	square centimetre . . ./centimètre carré . . .	cm^2 . . .
volume/volume	millilitre, litre . . ./millilitre, litre . . .	mL, L . . .
time/temps	second/seconde	s
	minute/minute	min
	hour/heure	h
	day/jour	d
frequency/fréquence	hertz/hertz	Hz
mass/masse	gram, kilogram . . ./gramme, kilogramme . . .	g, kg . . .
density/masse volumique	kilogram per cubic metre/kilogramme par mètre cube	kg/m^3
pressure/pression	pascal/pascal	Pa
temperature/température	degree Celsius/degré Celsius	°C
electric current/courant électrique	ampere/ampère	A
conductance/conductance	siemen/siemen	S
luminous intensity/intensité lumineuse	candela/candela	cd
illuminance/éclairement	lux/lux	lx
amount of substance/quantité de matière	mole/mole	mol
molar concentration — volume/ molalité concentration — volume	mole per cubic metre/mole par mètre cube	mol/m^3
molar concentration — mass/ molalité concentration — massique	mole per kilogram/mole par kilogramme	mol/kg
ppm (parts per million)	microgram per gram/microgramme par gramme	$\mu g/g$
	milligram per litre/milligramme par litre	mg/L
	microlitre per litre/microlitre par litre	$\mu L/L$
ppb (parts per billion)	nanogram per gram/nanogramme par gramme	ng/g
	microgram per litre/microgramme par litre	$\mu g/L$

The following units are not acceptable in the SI and authors using them are asked to give SI equivalents. If both units are given it is preferable to put the non-SI one in parentheses.

Les unités qui suivent ne sont pas acceptées par le SI et les auteurs qui les utilisent sont priés de donner l'équivalent. Si les deux unités sont données, il est préférable de placer entre parenthèses celle qui n'est pas du système SI.

Not acceptable in SI/ Non valides dans le SI	SI equivalents/Équivalents SI	Conversion factors/ Facteurs de conversion
ångström/ångström (Å)	nanometre/nanomètre (nm)	1 Å = 0.1 nm
micron/micron (μ)	micrometre/micromètre (μm)	1 μ = 1 μm
calorie/calorie (cal)	joule/joule (J)	1 cal = 4.1868 J
inch (in)/pouce (po)	centimetre/centimètre (cm)	1 in/1 po = 2.54 cm
foot (ft)/pied (pi)	metre/mètre (m)	1 ft/1 pi = 0.305 m
mile/mille (mi)	kilometre/kilomètre (km)	1 mi = 1.6 km
curie/curie (Ci)	becquerel/becquerel (Bq)	1 Ci = 37 GBq

Publications diverses spéciales

L'Homme, les lacs et la prolifération des algues

J. R. VALLENTYNE

(1978, 196 p., N° de catalogue Fs 4-31/22F; Canada $3.75, autres pays $4.50)

Cet ouvrage, préparé à l'intention des non-spécialistes, traite des lacs et de la façon dont ils sont affectés par les apports de matières nutritives découlant des activités humaines (eutrophisation due à l'Homme). Alors que le thème central de l'ouvrage porte sur le phosphore en tant que clé de la lutte contre l'eutrophisation due à l'Homme, une analyse des causes réelles fait ressortir la nécessité de limiter la croissance démophorique. Le lecteur trouvera en fin de volume un glossaire des termes scientifiques, des références suivies d'une liste de lectures supplémentaires ainsi qu'un appendice comprenant un bilan de la situation en 1974 et une série de tableaux et de figures.

Guide pratique de l'anatomie et de la physiologie du saumon du Pacifique

L. S. SMITH ET G. R. BELL

(1978, 15 p., N° de catalogue Fs 4-31/27F; Canada $2.50, autres pays $3.00)

La présente publication, illustrée de dessins et de photographies, contient une description de l'anatomie macroscopique des saumons du Pacifique. Les aspects de l'anatomie et de la physiologie qui présentent un intérêt particulier pour les biologistes des pêches y sont soulignés. De plus, le système cardio-vasculaire est étudié en détail à l'aide d'angiogrammes et de pièces moulées en plastique rigide du système sanguin. On ne discute que brièvement de l'anatomie du squelette, des muscles et du système nerveux. Notons que presque toute cette information s'applique également au saumon atlantique et aux truites.

Procédés de traitement et de conservation du maquereau. Étude et bibliographie annotée.

J. R. DINGLE

(1978, 69 p., N° de catalogue Fs 4-31/30F; Canada $3.00, autres pays $3.60)

La présente publication rassemble la documentation technique et scientifique parue avant 1974 et ayant trait au maquereau dans l'alimentation humaine. Il comprend 184 résumés ainsi qu'une liste de sujets choisis.

Ces publications sont en vente à

Imprimerie et Édition
Approvisionnements et Services Canada
Ottawa K1A 0S9

Les chèques ou mandats-postes, payables à l'avance en monnaie canadienne, doivent être faits à l'ordre du Receveur général du Canada.

Journal of the Fisheries Research Board of Canada

Journal de l'office des recherches sur les pêcheries du Canada

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